PHASE II TRIAL OF ESTROGEN RECEPTOR TARGETED TREATMENT OF NON-MUSCLE INVASIVE BLADDER CANCER WITH TAMOXIFEN (05-09-10-02)

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This clinical research protocol will be conducted in accordance with FDA, ICH and IRB regulations and guidelines. The Scott Department of Urology complies fully with the HIPAA guidelines.

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Rationale/Background:

BACKGROUND AND SIGNIFICANCE

The American Cancer Society's most recent estimates regarding bladder cancer in the United States are that there will be 74,690 new cases with 15,580 deaths in 2014. The chance of a man having this cancer is about 1 in 26. For women, it is 1 in 87. In men, bladder cancer is the fourth most common and eighth most lethal of all malignancies.[1] Bladder cancer is more common among men than women and among whites than blacks. This is a cancer of older people, as nearly 90% of people with this cancer are over the age of 55. The earlier this cancer is found and treated, the better the outcome. Bladder cancer is a heterogeneous group of tumors with different biologic behaviors. The majority (75-80%) are non-muscle invasive urothelial carcinomas, which frequently recur (>50%), progress to an invasive phenotype in 10-15% of patients, but seldom metastasize.[2] Of all cancers, bladder cancer has the highest cost per patient from diagnosis to death and has the fifth highest cost for total medical care expenditures.[3]

Defining disease stage, grade and risk-groups

The current American Joint Committee on Cancer (AJCC) TNM stage system for bladder tumor classifies papillary lesions according to their degree of invasion in the bladder wall layers. Tumors that are confined to the urothelium or invade only the underlying lamina propria but not the muscularis propria, are generally called non-muscle invasive tumors. These tumors can be classified as carcinoma in situ or Tis (flat lesions, with epithelial abnormalities only), Ta (papillary lesions that do not penetrate the basement membrane) or T1 (tumors that invade only into the lamina propria). These lesions are also classified in a dichotomous fashion according to their degree of dysplasia as low-grade (LG) or high-grade (HG), as defined by the World Health Organization/ International Society of Urological Pathology (WHO/ISUP) 2004 classification.4 Patients with Tis and/or HG Ta or T1 have an increased risk for recurrence and progression to a muscle invasive cancer.[5] *Recurrence* is defined in this setting as appearance of a tumor in the bladder following the initial treatment, in the same or inferior stage and/or grade, whereas *progression* is defined as recurrence of a tumor of higher stage and/or grade. The other lesions (Ta-1LG) are classified as low- or intermediate-risk for recurrence or progression. This risk stratification is based upon the tumor size (≥3cm vs. <3cm), the number of lesions (2 or more, vs. single), and the number of previous recurrences (recurrent vs. primary).[6] Non-invasive bladder tumors in the intermediate-risk category have recurrence rates ranging from 24-38% and 46-62% in 1 and 5 years, respectively. Their rates of progression are about 1% and 6% in 1 and 5 years, respectively. Comparatively, patients in the low-risk category have recurrence in 15% and 31% of cases, but have progression only in 0.2% and 0.8% of the times, at 1 and 5 years, respectively.[6] Therefore, the low/intermediate-risk groups of tumors deserve the attention of this study because the risk of progression, albeit low, is relevant and the recurrence risk is high, even with optimized intravesical chemotherapy.[7]

Status of currently available therapies

There currently are no oral or intravenous therapies for the non-invasive group of bladder tumors. Current treatment strategy is based on surgical transurethral resection (TUR) of the bladder tumor and the application of intravesical chemotherapy. Drugs applied intravesically can be administered in three different regimens:

A) Immediate Post-Operative: Usually a single instillation within the first 6 hours after the TUR.

B) Induction Course: Comprised of a series of 4-6 weekly instillations started within 2-4 weeks after the TUR.
C) Maintenance Course: Usually given in a more delayed and prolonged fashion, usually in a monthly schedule over the period of one year. The maintenance course starts 6 weeks after completion of the induction, between the second and third month after the TUR, and usually includes 10 monthly intravesical instillations.

There is controversy about the ideal regimen and chemotherapy drug to be recommended for each risk group, but for low/intermediate-risk bladder tumors, it is usually offered a single dose of mitomycin-C in the immediate post-operative period, followed by an induction course for 6 weeks using an optimized drug delivery strategy.[2, 7-10] There may also be a rationale for monthly maintenance single dose intravesical chemotherapy for up to one year after the induction course, i.e. 10 additional monthly intravesical instillations.

A variety of chemotherapeutic agents given by intravesical administration have been used in attempts to reduce the probability of recurrence and progression. The main advantage of this route of administration is the high intravesical concentration achieved, facilitating absorption into the bladder mucosa, with minimal risk of systemic absorption, and thus limitation of the risk of systemic toxicity. Valrubicin, thiotepa, mitomycin-C, doxorubicin, gemcitabine, ethoglucid and teniposide have been used with varying degrees of success for different risk-groups of non-invasive bladder tumors. Long-term recurrence rates are still high despite inclusion of induction and maintenance treatment regimens. Repeat treatments may be effective, yet may be associated with an increased risk of local and systemic side effects and increased costs and bother.

Pharmacologic optimization of intravesical mitomycin-C consists of several procedures that aim at optimization of the urine pH and drug concentration, in order to improve the local effect of the chemotherapy agent when administered intravesically.[7] Although the implementation of the optimized protocol improves the median time to recurrence, the associated tumor-free probability at 5 years remains at 41%.[7]

In summary, there is a need for new therapeutic strategies, using effective and well tolerated drugs that improve overall outcomes while reducing costs, invasiveness, and burden of the disease with minimal toxicity. Chemopreventive treatments are needed in our therapeutic armamentarium, both for maintenance of the initial response to treatment and for the potential for long-term prevention of recurrence and progression. Additionally, there is a need for biomarkers that can be used to predict treatment response before therapy is initiated and also to non-invasively monitor the response during the course of therapy.

Estrogen receptors and their biological role

Estrogen promotes the growth of a number of tissues (e.g. breast and uterine epithelium) and can also stimulate the growth of tumor cells in these tissues. Two ER subtypes, ERα and ERβ, mediate the physiologic response to estrogen and exhibit separate and overlapping tissue distributions and functions. Unlike in normal and neoplastic breast, where the major effector appears to be ERα, in the urinary tract the predominant receptor is ERβ, although its biologic role is not completely defined.[11-13]

Rationale for targeting ER

The finding that ERβ knock-out mice exhibit increased prostate and bladder hyperplasia suggests a potential role of ERβ in the development of urologic cancers.[14] While ERα is expressed in 12.4%-18% of bladder cancers[15,16], we have found that ERβ is the predominant receptor expressed in several human bladder cancer cell lines and human bladder cancers comprised in tissue microarrays.[17] An independent report from separate human tissue microarrays, analyzing a heterogeneous group of bladder tumors including different stages and grades from 2 institutions, also confirmed the expression of ERβ to be higher than ERα, and significantly higher expressed in high-grade/muscle-invasive tumors in comparison with low/non-muscle-invasive tumors.[18] These data suggest that ER may be a rational target for intervention with selective estrogen receptor modulators (SERMs) for treatment and chemoprevention of bladder cancer.

Rationale for using SERMs and tamoxifen in bladder cancer

Tamoxifen, the prototypic SERM, has been utilized for breast cancer treatment for thirty years and more recently for primary and tertiary chemoprevention of breast cancer. Through its interactions with both ER types, it functions as an antagonist in the breast and an agonist in bone and uterus, also reducing circulating cholesterol levels. In breast cancer, the response to tamoxifen generally correlates with ER expression.[19] In addition, ERβ modifies the action of ERα and reduces the estrogen-like effects of tamoxifen.[20] It is likely that the relative expression levels of the two forms of the ER and its splicing variants will be a key determinant of cellular responses to SERMs with differing agonist and antagonist properties.[20] It is also possible that tamoxifen may modulate ER expression. While tamoxifen has been shown to increase ERα expression in vitro in breast cancer cells, tumors may lose ER expression and become resistant to anti-estrogen therapy.[21,22] Further evidence of the importance of ERβ is demonstrated by women with ERα negative breast cancer treated with tamoxifen, showing better survival associated with ERβ immunoreactivity levels.[23] ERβ may therefore confer a protective effect in the absence of ERα, retaining sensitivity to therapy using SERMs. The TSU-PR1 bladder cancer cell line, which expresses a high level of ERβ but not ERα, is sensitive to tamoxifen.[24] Our studies of SERMs in pre-clinical animal models demonstrate that these agents have a potent inhibitory effect on experimental human bladder cancer tumor growth, providing proof of concept of their potential use as therapeutic agents for human bladder cancer.[17, 25] SERMs exert their anti-tumor effects in part via inhibition of proliferation and induction of apoptosis, which correlate with response to treatment. Several urine biomarkers have been shown to correlate with stage and grade of bladder cancer, and the presence or absence of tumor. In addition, persistence of urine biomarker after resection with or without intravesical chemotherapy suggests persistence of occult disease and is frequently associated with a higher risk of subsequent tumor recurrence.[26-28] Therefore, it is reasonable to expect that if tamoxifen modulates the growth of bladder cancer, this will be reflected by the chosen biomarkers for apoptosis and cell proliferation.

Rationale for investigating the role of ERβ splicing variants in human bladder cancer

Multiple splicing variants of both ERα and ERβ have been described in various tissues.[29-32] The controversy over the function of ER as promoter of carcinogenesis in some tissues and immuno-modulator in others may be related to the significant expression of splicing variants.[33, 34] New emerging evidence has suggested that these isoforms are expressed in significant amounts and may be related to different heterogeneous effects obtained by the interaction of these variants forming dimers with the full-length receptors, modulating their action and also binding to

different ligands in the tissues, including estrogens and SERMs.[31, 33, 34] The understanding of the role and mechanisms through which these receptors interact with their ligands is still in its infancy, but knowledge about the wild type (ERβ1), and also ERβ2 and ERβ5, two of the most commonly studied variants, is growing fast.[31, 35, 36] More recent studies in human and animal models of breast and prostate cancer have shown that these splicing variants are expressed more frequently than the wild type in some tissues and that they are not independently functional as homodimers, but they make heterodimers with the wild type ERβ, thus modulating its activity.[36-42]

Because our aim #2 is to explore the expression of the human ERs in normal and tumor samples from the bladder, and also their potential modulation after exposure to a SERM drug, these findings will also provide an unique opportunity to report the expression levels of the most common splicing variants in human bladder tissue. Because ERβ is more common than ERα in the bladder, we will only study the ERβ splicing variants. Thus, for the purpose of this study, we will evaluate only ERα, and ERβ1 (wild type), ERβ2 and ERβ5.

Overall Significance

Non-invasive bladder cancer is challenging to treat because it is a condition characterized by frequent recurrences that lead to numerous invasive procedures for detection and treatment. The mainstay of therapy for non-muscle invasive bladder cancer, which has remained unchanged for decades, relies on surgical resection (TUR) associated with utilization of intravesical immunotherapy and chemotherapy. Despite our best efforts, these tumors continue to have suboptimal rates of recurrence and require long-term follow-up with costly and invasive procedures. The results of the present study have the potential to improve the oncological outcomes, and at the same time to create a new paradigm in the management of bladder cancer, with the introduction of an oral agent to the current armamentarium. Additionally, with less frequent recurrences and improved biomarkers, this study has the potential to reduce the burden, the costs, and the invasiveness of current treatment and follow-up strategies.

Hypotheses:

Oral tamoxifen treatment will:

1) Reduce the size or eliminate small bladder tumor marker lesions, by induction of apoptosis in the tumor cells, being well tolerated with minimum toxicity.

2) Show a positive correlation between the clinical response and the pretreatment expression levels of ER (especially ERβ and its splicing variants) in the tissues.

Specific Aims:

Aim 1. Evaluate the efficacy of tamoxifen for treatment of low/intermediate-risk bladder tumors, assessing for the clinical response of the marker lesion, and analyzing the effects in tissue, in terms of affecting apoptosis and proliferation, while continuously monitoring for treatment toxicity.

1a. Evaluate the efficacy of tamoxifen for treatment of low/intermediate-risk bladder tumors, utilizing the RECIST criteria combined with the final biopsy of the marker lesion or the bed of the lesion in case of a complete response. (PRIMARY ENDPOINT)

1b. Evaluate the histopathologic effect of tamoxifen treatment in the tumor cells, assessing for the presence of necrosis, fibrosis, apoptosis and scar tissue.
1c. Evaluate the treatment effect in the histologic markers for apoptosis (TUNEL) and proliferation (Ki-67).
1d. Monitor treatment toxicity.

Aim 2. Evaluate the expression levels of ERα and ERβ (including splicing variants) before and after treatment with tamoxifen, in both normal and tumor tissues, and correlate the clinical response with the expression levels of these ERs.
2a. Report on and compare the pre and post-treatment expression levels of ERα and ERβ (including their splicing variants, more specifically ERβ-1, ERβ-2 and ERβ-5) in both normal and tumor tissues.
2b. Correlate the observed clinical effect, measured by the proportion of subjects that had complete or partial responses, with the pretreatment expression levels of ERs in tumor tissue.

Primary objective:
Evaluate the efficacy of tamoxifen for treatment of low/intermediate-risk bladder tumors, assessing for the clinical response of the marker lesion.

Secondary objectives:
While assessing for clinical response of the marker lesion, we will also analyze the effects in tissue, in terms of affecting apoptosis and proliferation, while continuously monitoring for treatment toxicity.
Additionally, we will evaluate the expression levels of ERα and ERβ (including splicing variants) before and after treatment with tamoxifen, in both normal and tumor tissues, and correlate the clinical response with the pretreatment expression levels of these ERs.

Drug information:
A. Tamoxifen
The manufacturer states that the adverse effect profile of tamoxifen in breast cancer patients generally appears to be similar for men and women. Adverse effects of tamoxifen citrate usually are relatively mild and rarely severe enough to necessitate discontinuation of the drug in patients with breast cancer. Tamoxifen usually is well tolerated in male patients with breast cancer. The main potential adverse effects reported in patients receiving tamoxifen are:
1. Cardiovascular effects, including thrombotic and venous thromboembolic events such as stroke, pulmonary embolism, and deep-vein thrombosis, fluid retention and edema.
2. Vasomotor symptoms (i.e. hot flushes).
3. Effects on lipoproteins (hyperlipidemias and hypertriglyceridemia).
5. Genitourinary and renal effects (vaginal discharge and menstrual irregularities, loss of libido and impotence, increased serum BUN and/or creatinine).
6. Musculoskeletal and bone pain, and hypercalcemia.
7. Ocular effects (retinopathy, corneal opacities, and decreased visual acuity).
8. Hepatic effects (increased serum AST (SGOT) or ALT (SGPT) concentrations, and increased bilirubin and/or alkaline phosphatase concentrations).

9. Gastrointestinal effects (nausea, anorexia, distaste for food, and abdominal cramps).
10. Nervous system effects (dizziness, lightheadedness, headache, fatigue, and mental depression).
11. Hematologic effects (thrombocytopenia and neutropenia, pancytopenia, and leucopenia).
12. Dermatologic effects (thinning and/or partial loss of hair).
13. Other Adverse effects (weight loss, fatigue, and cough).
14. Carcinogenicity (increased incidence of uterine cancer).
15. Pregnancy and fertility (tamoxifen may cause fetal harm when administered to pregnant women. Effects on reproductive function are expected from the anti-estrogenic properties of the drug).

Phase of study: *S*ingle-center, two-stage phase-II clinical trial (Simon design)

Eligibility criteria:

Inclusion

1. Males and females age 21 or older.
2. Histologic evidence of urothelial carcinoma of the bladder.
3. Low/Intermediate-risk papillary urothelial carcinoma of the bladder, at initial occurrence or recurrent with >6 months interval free of disease.
4. Patients with multifocal tumors must have resectable lesions.
5. Patients may be treatment-naïve or have failed previous regimen of intravesical therapy.
6. At least one endoscopically measurable tumor 6 - 10mm in diameter.
7. Adequate hepatic and renal function normal ranges defined as:

Creatinine 0.6-1.3 MG/DL
BUN 10-20 MG/dL
AST (SGOT) 5-35 U/L
ALT (SGPT) 7-56U/L
GGT 0-45U/L

8. Patient or authorized proxy needs to have signed the informed consent form.

General Exclusion

1. Patients with sessile appearing tumors, which may be invasive or high-grade.
2. Diagnosis of urothelial carcinoma involving the prostatic urethra or upper urinary tract.
3. Plans for pelvic radiation while participating in the study.
4. Concurrent use of selective serotonin reuptake inhibitors or aromatase inhibitors.
5. Chronic or acute renal or hepatic disorder or any other condition, medical or psychological that, in the opinion of the investigator, could jeopardize the subject's safe participation.
6. Any other investigational drug within 30 days prior to registration and during the study.
7. Any subject who, in the opinion of the PI is at risk for thromboembolic events.
8. Any patients taking drugs that are known to have drug interactions with Tamoxifen will not be included.

Women Exclusion

1. Pregnant or lactating women.

2. Personal history of endometrial cancer or any abnormal uterine bleeding.
3. Previous or concurrent treatment with SERM and/or hormonal replacement therapy within 3 months of the study.

Design:

Study design

We will conduct a single-center, two-stage phase-II, clinical trial utilizing the marker lesion study design, where a tumor is mapped in the bladder, treated, and re-assessed after the study intervention to evaluate the effects of the therapy. This study design will allow the evaluation of the size of the tumor before and after treatment, and the histological assessment of treatment effect in normal and tumor tissues, as well as modulation of immunohistochemistry markers for apoptosis and proliferation, and of the expression levels of ERα, ERβ1, ERβ2, and ERβ5.

Overall study description

Patients with primary or recurrent low/intermediate-risk papillary urothelial carcinoma of the bladder will undergo resection of all but one marker lesion, measuring at least 6mm but no greater than 10mm, and biopsy of normal-appearing mucosa. Patients with a solitary tumor will undergo only biopsy prior to treatment. A 2mm cold cup biopsy of the marker lesion will always be performed to rule out a potential high-grade lesion and for assessment of pretreatment immunohistochemistry expression levels of ERα, ERβ1, Ki-67 (proliferation marker) and TUNEL (apoptosis marker). If there are multiple tumors, all lesions, except the marker lesion will be resected and sent for analysis. These patients will not receive single immediate post-operative intravesical instillation of mitomycin-C. They will then undergo a 12-week course of treatment with tamoxifen administered as a single daily oral dose of 20mg. At the completion of therapy, patients will undergo resection of the marker lesion (or biopsy of the tumor bed, if a complete response is observed) and biopsy of normal-appearing bladder mucosa again. Toxicity evaluations will be performed at the beginning (day 3), midway (week 6), and at completion of treatment (week 12), prior to resection of the marker lesion. A final assessment for toxicity will also be performed 30 days after completion of therapy as well as a second definitive resection of the marker lesion. Urine samples will be obtained with the index tumor in place (marker lesion), and after completion of treatment, at the time of definitive transurethral resection of the index tumor, as part of the standard clinical care of these patients, and at the discretion of the surgeon for assessment of urinary cytology. The urine samples will not be utilized for the research study. All normal-appearing bladder biopsies (pre and post-treatment), the additional tumors (in case of multiple lesions), and the definitive resection of the marker lesion (in the absence of response to therapy) will provide sufficient material for immunohistochemistry assessment of the expression levels of ERα, ERβ-1, ERβ-2, ERβ-5, Ki-67, and TUNEL, and also for RT-qPCR for mRNA analyses of ERα, ERβ-1, ERβ-2, and ERβ-5. The pretreatment biopsy of the marker lesion will be performed with a smaller biopsy forceps and will provide limited material, sufficient only for immunohistochemistry assessment of the expression levels of ERα, ERβ-1, Ki-67, and TUNEL.

Marker lesion study design

The marker lesion study design consists of deliberately leaving a small visible lesion in the bladder, so the patient can receive the study treatment, and shortly afterward have the lesion

removed and re-assessed for evaluation of treatment efficacy. Extensive natural history studies observing small low/intermediate-risk bladder tumors demonstrate that they grow slowly with very minimal risk of progression, and that they usually remain stable in size for at least 12 weeks without exhibiting spontaneous remission. The marker lesion should be papillary, since sessile tumors are likely high-grade, and should measure 6-10 mm in diameter. Patients with multiple tumors will have all visible disease resected with the exception of the marker lesion. A single small cold cup biopsy will be taken of the marker lesion, and a pilot study (H-33285) is being completed to prove that biopsying the marker lesion does not cause necrosis, fibrosis and scarring that can lead to confusion or mask either the primary or secondary endpoints. Because the natural history of this category of bladder lesions is so well-known and extensively described, there is no need for a control group in this study.

Collection of specimens

A random biopsy of normal appearing mucosa remote from any tumor will be required pre and post-treatment. All immunohistochemistry studies will be performed on formalin-fixed paraffin-embedded tissue. Tissue destined to mRNA analysis will be directed to the research laboratory for analysis. . At the end of the trial, these specimens will be destroyed in an appropriate manner, according to the BCM rules and regulations.

Study Schedule

Tests/Time	Screening	1st Resection Tamoxifen starts	3 days after treatment initiation +/- 2 Days	6 weeks after treatment initiation +/- 2 Weeks	2nd Resection (week 12) 1-2 Weeks after stopping Tamoxifen	Follow-Up Tamoxifen ends	30 days Post- 2nd Resection +/- 1 Week	Follow-Up Visit 3 months Post- 2nd Resection +/- 1 Week
Informed consent	X							
Eligibility Criteria	X							
History/Physical Exam**	X							X###
Office Visits#	X	X			X			X###
Hematology/Phlebotomy**	X							X###
CBC/platelets**	X							X###
Bilirubin**	X							X###
Urinalysis**	X							X###
Urine pregnancy test, if needed	X							X###
Cystoscopy	X	X			X			X
Cytology**	X	X			X			X
Voided FISH**		X						X
Bladder Biopsy- Index tumor TURBT - measurement Primary Endpoints		X			X			
Drug dispensing/accountability*		X			X			
Tamoxifen – oral 20mg/day*		X	X	X	X			
Concomitant Meds	X	X	X	X	X	X	X	X
Adverse Events (NCI CTC v.4.0)	X	X	X	X	X	X	X	X

Footnotes: (Standard of care events/procedures are shaded):

* Tamoxifen administered at 20 mg/day as a single daily oral dose for 12 weeks

**Cytology and FISH within 1 month prior to Screening/Day 1 is acceptable

*** For Eligibility, Labs and Physical Exam can be performed within 3 months prior to Screening/Day 1

Office visit on 2nd Resection only occurs if patient had a Single Tumor during 1st Resection. All patients with Multiple tumors at 1st Resection will have 2nd Resection in an outpatient hospital setting.

Completed per PI descretion

Procedures:

Aim 1 Procedures: Transurethral resection of bladder tumors is a well-defined surgical technique used for diagnosis, staging, and treatment of bladder tumors of different stages. Briefly, patients undergo general anesthesia. Under proper aseptic technique, an optical resectoscope with video imaging capabilities is introduced into the bladder retrogradely via the urethra. The bladder tumor is resected using a resection loop connected to an electric source. The technique involves scooping movements of the loop, while cauterization is applied, to allow concomitant tissue cut and hemostasis. Measurement of the lesions will be performed by comparison with the standardized size of the resection loop.

Collection of samples: Biopsies of the marker lesion and normal appearing mucosa located remotely from the marker lesion will be processed using standard procedures for formalin fixation and paraffin embedding. These tissues will be tested by immunohistochemistry for Ki-67 and TUNEL. Each specimen will also be stained with hematoxylin and eosin to verify urothelial carcinoma histology.

Aim 2 Procedures: Collection of samples: Biopsies of the marker lesion and normal mucosa remote from the marker lesion will be processed using standard procedures for formalin fixation and paraffin embedding. The pretreatment biopsy of the marker lesion will be performed with a 2mm cold-cup biopsy forceps, to avoid affecting the histologic characteristics of the marker lesion. Because of the limited amount of tissue yield in this biopsy, we will only perform immunohistochemistry analyzing Ki-67, TUNEL, ERα and ERβ-1. The normal bladder biopsies, the resection of additional tumors synchronous to the marker lesion (in case of multiple lesions), and the final definitive resection of the marker lesion will be performed with a regular rigid biopsy forceps or resection loop, yielding much larger specimen fragments. These tissues will be tested by immunohistochemistry and RT-qPCR for the mRNA of ERα, ERβ-1, ERβ-2 and ERβ-5. Each specimen will also be stained with hematoxylin and eosin to verify urothelial carcinoma histology.

Samples Concerns

A urine specimen will be collected for cytology and pregnancy testing. The specimen will be discarded as per routine destruction methods.

The tissue collected from the bladder lesions will be processed and assessed for the immunohistochemistry and RT-qPCR tests in Dr. Seth Lerner's laboratory at Main Baylor, Room 506C. Analysis will include normal H&E pathological examination and immunohistochemistry for Ki-67, TUNEL, ERα and ERβ-1, ERβ-2 and ERβ-5 will be performed, and RT-qPCR analyses will be performed for ERα, ERβ-1, ERβ-2 and ERβ-5.

Approximately two teaspoons of blood will be drawn for the analysis of CBC, platelets and bilirubin. Samples are destroyed as for any standard of care blood work. No cell lines will be developed with the specimen samples obtain during this study. No blood samples will be taken for any research study purpose.

The biopsy samples will be coded and labeled with a 3 digit number starting with 001, 002. This study number will only be linked to the clinical information by a log file, stored safely in the Department of Urology computer servers, protected behind BCM firewall and individual password protected system access. Only the PI and the authorized study staff will have access to this log file, being able to link the clinical information with the specimen numeric codes.

These specimen tissue samples will not be released to other hospitals or laboratories, and they will be used exclusively for regular clinical purposes and the research procedures described in this protocol.

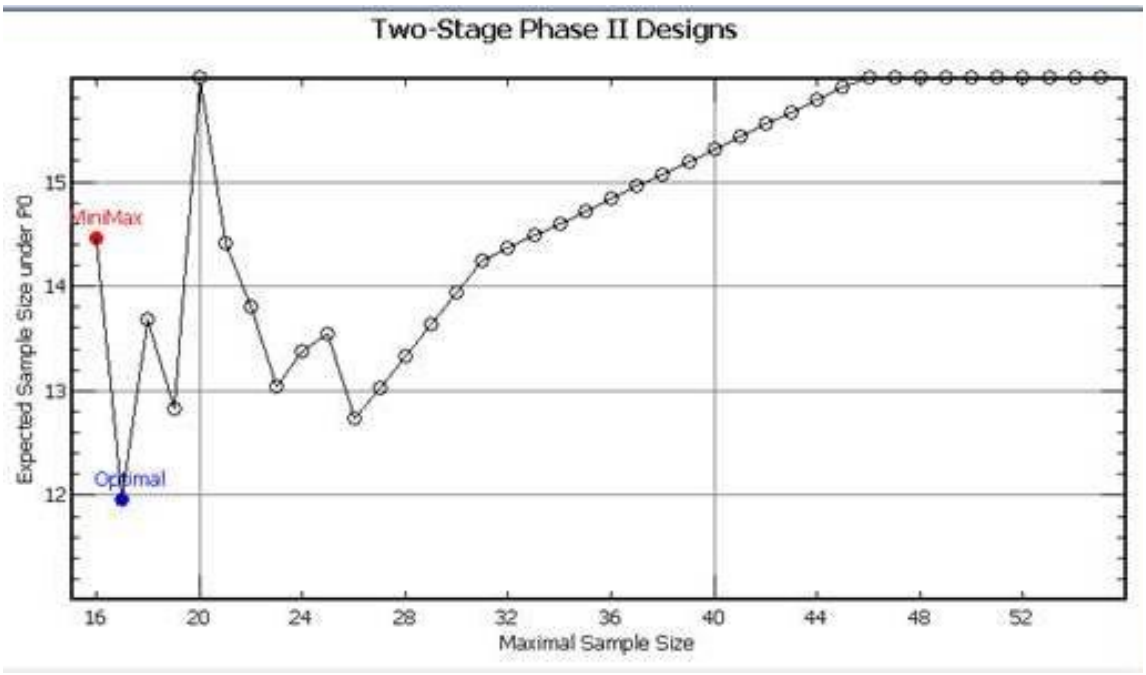
These specimen tissue samples will not be banked for future use. If there is a remaining sample after the planned analyses are performed, these specimens will be kept until the completion of the study These samples will not be available to other research projects for other testing.

If the study subject withdraws from the study or revokes authorization in writing, he/she will be removed from the study, but the remaining specimens will not be returned to the subject but will be destroyed upon written request sent to Dr. Godoy. The data collected prior to destruction will not be deleted.

The study results will not be recorded in the subjects' medical records, because this information has no validated clinical meaning; therefore, no clinical use currently. For the same reason, the results from this study will not be revealed to the research study subject or his/her doctor who is not included in this study protocol. Subjects may never be able to obtain limited research health information.

Sample Size:

This is a single-arm, two-stage phase-II trial (Simon design) to demonstrate that treatment with 20 mg/day of oral tamoxifen for 12 weeks is associated with elimination or a greater than 30% reduction in size of a bladder cancer marker lesion from pre to post-treatment.



The table above shows the Simon's two-stage phase-II study design plot, assuming that the bad response rate (P0) is 0.05, the good response rate (P1) is 0.30, significance level (α) is 0.05, power is 0.90, and confidence interval is 95%.

The optimal design was chosen to minimize the expected sample size, and reduce the number of subjects enrolled if the study is negative. According to this method, the maximum sample size is 17 and the minimum sample size is 9. The first stage of the study will include 9 patients. If there are 0 responses observed, the trial will be stopped accepting H0. If not, the trial continues to the stage-two, accruing 8 more patients (total of 17). If there are 2 or fewer total responses observed, or 3 or more total responses observed, then we will accept or reject H0, respectively.

Considering a 10% drop-off rate, the expected maximum sample size needed for this study will be 19 subjects, so we are able to accrue 17 evaluable subjects. Evaluable subjects meet all the eligibility criteria and receive at least 30 days of Tamoxifen. All subjects who take at least one dose of Tamoxifen will be evaluable for safety. Since the past five-year median accrual rate for this group of patients in our center has been 10-12 cases/year, we expect conservatively to complete accrual in 2 years.

Data Analysis: See below under End-points/Statistical considerations

End-points/Statistical considerations:
The primary outcome of interest is a composite endpoint, defined according to the RECIST (Response Evaluation Criteria in Solid Tumors) criteria. The response criteria evaluation of target lesions is defined as follows:
- *Complete Response (CR):* Disappearance of all target lesions;

- *Partial Response (PR):* At least a 30% decrease in the longest diameter of target lesions, taking as reference the baseline longest diameter;
- *Progressive Disease (PD):* At least a 20% increase in the longest diameter of target lesions, taking as reference the baseline longest diameter recorded or the appearance of one or more new lesions;
- *Stable Disease (SD):* Neither sufficient shrinkage to qualify for PR, nor sufficient increase to qualify for PD, taking as reference the baseline longest diameter.

The endpoint will be considered a positive response if the endoscopic outcome is a CR or PR, or the biopsy of a residual tumor or the bed of the lesion (in case of a CR) is negative for bladder cancer. If biopsy of the marker lesion at the end of the study is positive for bladder cancer, and according to RECIST criteria, the outcome is PD or SD, the treatment response to tamoxifen therapy will be considered negative. The table below displays the summary of endpoints.

	Cystoscopy	Biopsy	Final Overall Response
Positive response	CR	negative (bed)	CR → success
	PR/SD	negative	CR → success (no viable tumor with necrosis, fibrosis, or scar tissue)
	CR	positive (bed)	PR → success
	PR	positive	PR → success
	PD	negative (unlikely)	CR → success
Negative response	PD	positive	PD → failure
	SD	positive	SD → failure

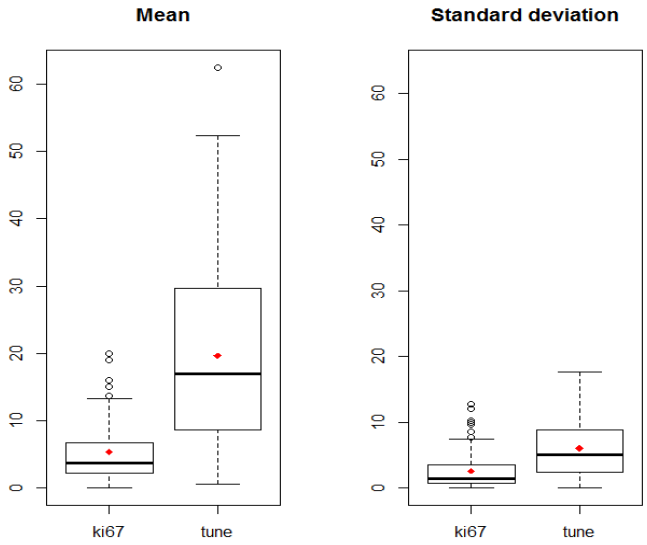
Notes: CR=complete response; PR=partial response; SD=stable disease; PD=progressive disease.

Size will be measured in millimeters and analyzed as a composite dichotomous endpoint as defined above. For this trial, we will include patients with marker lesions ranging in size from 6 to 10 mm. As shown by data from our pilot study discussed earlier, we are able to measure these lesions accurately, with a standard deviation of 1.5mm, and we are able to statistically detect 15% reduction in size of these lesions.

Statistical Considerations for Biomarkers:
Preliminary data was analyzed on a retrospective series extracted from previously built tissue microarrays with human bladder cancer specimens at various stages. For this analysis we selected 108 and 99 patients with stage Ta and T1 on each biomarker, Ki-67 and TUNEL, respectively.

Stage	n	
	Ki-67	TUNEL
TIS	1	1
TA	78	73
T1	29	25
T1A	1	1
T2A	11	8
T2B	3	2
T3A	22	10
T3B	13	8
T4A	5	3
T4B	2	1
NA	1	1
Total	166	133

Mean expression with standard deviation for each subject with Ta or T1 disease with more than one core (Ki-67, n=82; TUNEL, n=65) was computed. These data are presented in the boxplots below. Median values of these means and standard deviations were used in the calculation of power and sample size. In the plot, the red mark is the mean and the horizontal line is the median.



Calculation of sample size and power for Ki-67 analysis
The maximum sample size of 17 will have 85.6% power to detect a difference in means of 30% (-1.101 or 1.101, e.g. a first condition mean, μ1, of 3.670 and a second condition mean, μ2, of 2.569), assuming a standard deviation of differences of 1.41, using a paired t-test with a 0.050 two-sided significance level. Alternatively, the minimum sample size of 9 will have 51.5% power. With the maximum sample size, a 27.8% detectable difference (-1.021 or 1.021) could be significantly identified under α=0.05 and power=0.8 assumptions.

Significant level (2-sided)	0.05	0.05	0.05
First condition mean	3.67	3.67	3.67
Second condition mean	2.569	2.569	2.613
Mean difference	1.101	1.101	1.021
Standard deviation of differences	1.41	1.41	1.41
Power (%)	85.6	51.5	80.0
N	17	9	17

Ki-67: table for paired t-test power and sample size results

Calculation of sample size and power for TUNEL analysis
The maximum sample size of 17 will have 96.7% power to detect a difference in means of 30% (-5.1 or 5.1, e.g. a first condition mean, μ1, of 17 and a second condition mean, μ2, of 22.1), assuming a standard deviation of differences of 5.13, using a paired t-test with a 0.050 two-sided significance level. Alternatively, the minimum sample size of 9 will have 74.1% power. With the maximum sample size, a 21.8% detectable difference (-3.71 or 3.71) could be significantly identified under α=0.05 and power=0.8 assumptions.

Significant level (2-sided)	0.05	0.05	0.05
First condition mean	17	17	17
Second condition mean	22.1	22.1	20.85
Mean difference	5.1	5.1	3.71
Standard deviation of differences	5.13	5.13	5.13
Power (%)	96.7	74.1	80
N	17	9	17

TUNEL: table for paired t-test power and sample size results

In summary, the Simon's optimal design, and the maximum sample size of 17 subjects, will yield an 85.6% and 96.7% power (two-sided, paired t-test with 5% alpha) to identify 30% change in the Ki-67 and the TUNEL assays, respectively. Similarly, the minimum sample size of 9 will yield 51.5% and 74.1% power, respectively. Modulation of pre vs. post-treatment biomarker endpoints will be summarized and compared using the paired t-test or nonparametric paired tests for quantitatively measured biomarkers, including Ki-67, and TUNEL. Independent analyses will likewise be employed to compare these biomarkers measured between normal mucosa and marker bladder tumor, such as the STUDENT T-Test or the non-parametric counterpart (Mann-Whitney test), as appropriate.We will assess Pearlson's correlation. We will also assess the correlations of pretreatment expression levels of ERα and ERβ (wild type and its isoforms) with rates of positive response. Descriptive statistics will be calculated to summarize these biomarkers at pretreatment, post-treatment, difference from pre to post-treatment, and percent change from pre to post-treatment. Histologic effect will also be considered as a secondary endpoint in determining response to therapy. Histologic effect in the lesion will be summarized descriptively using frequencies and proportions before and after treatment.

Statistical considerations on the proposed analyses of the secondary aims, including the biomarker experiments

Specific Aim 1b: We will assess the histologic aspect of both normal tissue and the bladder cancer marker lesion biopsies before and after treatment and compare for the presence of fibrosis, scarring, tumor necrosis and apoptosis. These data will be measured and quantified using light microscopy so percent positivity can be evaluated. Comparisons will be made between normal and tumor tissues (independent samples analysis), and also between pre and post-treatment on both normal and tumor tissues (paired samples analysis) to evaluate the effect with tamoxifen treatment. Non-parametric tests will be utilized as appropriate.

Specific Aim 1c: We will compare pre and post-treatment levels of immunohistochemistry staining levels of both the normal tissue and the marker lesion to detect changes in expression of TUNEL and Ki-67, associated with treatment (paired samples analysis). Nuclear immunoreactivity will be assessed by light microscopy and percent positive stained nuclei calculated. The percentiles will be analyzed as a continuous variable. Just like above, non-parametric tests will be utilized as appropriate.

The modulation of pre vs. post-treatment biomarker endpoints will be summarized and compared using the paired t-test or nonparametric paired tests as appropriate for quantitatively measured biomarkers, including Ki-67, and TUNEL. Independent analyses will likewise be employed to compare these biomarkers measured between normal mucosa and marker bladder tumor. We will

also assess the correlations of TUNEL and Ki-67 with rates of positive response. Descriptive statistics will be calculated to summarize these biomarkers at pretreatment, post-treatment, difference from pre to post-treatment, and percent change from pre to post-treatment. Histologic effect will also be considered as a secondary endpoint in determining response to therapy. Histologic effect in the lesion will be summarized descriptively using frequencies and proportions before and after treatment.

Specific Aim 1d: Toxicity data will be presented and summarized using descriptive statistics, tabulated using frequencies and percentages.

Specific Aim 2a and 2b: All data will be presented and summarized using descriptive statistics. The percentage of positive cells with nuclear immunoreactivity is recorded on a semi-quantitative scale: results are categorized as negative (< 10% cell labeling) or positive (≥ 10% cell labeling). Normal breast tissue and transfected cells with known ER will be used as positive control and normal tissues such as liver and spleen, as well as cells transfected with empty vectors will be used as negative controls.

We will correlate pretreatment ER levels with the proportion of the positive response (composite endpoint of clinical PR and CR, and a final biopsy) of the marker lesion to determine associations with treatment effect. Specifically, Pearson's or Spearman's correlation coefficients will be calculated to estimate and test for significant correlations between the expression level of the ERs and the primary endpoint. Comparison of ERs expression levels from normal and bladder tumor tissues measured prior to treatment will also be performed using independent sample tests as described above. Non-parametric tests will be utilized as appropriate.

Risks:

Tamoxifen

The manufacturer states that the adverse effect profile of tamoxifen in breast cancer patients generally appears to be similar for men and women. Adverse effects of tamoxifen citrate usually are relatively mild and rarely severe enough to necessitate discontinuance of the drug in patients with breast cancer. Tamoxifen usually is well tolerated in male patients with breast cancer.

1. Cardiovascular Effects
   Adverse cardiovascular effects of tamoxifen include thrombotic and venous thromboembolic events such as stroke, pulmonary embolism, and deep-vein thrombosis.

2. Thromboembolic Events
   In the BCPT (Breast Cancer Prevention Trial): women without a history of pulmonary emboli receiving tamoxifen had an approximately threefold increase in the risk of developing a pulmonary embolism (18 versus 6 cases; incidence rate of 0.75 versus 0.25 per 1000 women-years) compared with those receiving placebo. Although the difference was not significant, the risk of deep-vein thrombosis was increased (relative risk of 1.6) among women receiving tamoxifen versus placebo in the BCPT (35 versus 22 cases according to data available as of March 31,

1998). A similar increase in relative risk of deep-vein thrombosis was observed in women aged 49 years and younger as in women aged 50 years or older, although fewer events occurred in younger women. Although the difference was not significant, an increase in the incidence of stroke was observed in women receiving tamoxifen (38 events) versus placebo (24 events) in the BCPT (relative risk of 1.59 and 95% confidence interval of 0.93-2.77 according to data available as of March 31, 1998).

3. Vasomotor Symptoms
In clinical studies, vasomotor symptoms (i.e., hot flushes [flashes]) occur more frequently in patients receiving tamoxifen than in those receiving placebo. In the BCPT, hot flushes occurred in 81% of women receiving tamoxifen compared with 69% of those receiving placebo.

4. Effects on Lipoproteins
Hyperlipidemias have been reported in patients receiving tamoxifen. Hypertriglyceridemia has been reported in patients with breast cancer receiving tamoxifen and may be severe, particularly in patients with a known history of elevated triglyceride levels, such as those associated with familial hypertriglyceridemia.

5. Other Cardiovascular Effects
Fluid retention has been reported in women receiving tamoxifen as adjuvant therapy for breast cancer. Edema has also been reported in women with metastatic breast cancer receiving tamoxifen.

6. Genitourinary and Renal Effects
Vaginal discharge and menstrual irregularities occur more frequently in patients receiving tamoxifen than in those receiving placebo. Vaginal discharge, occurring in 55%, was one of the most common adverse effects reported among women receiving tamoxifen for the primary prevention of breast cancer in the BCPT. Loss of libido and impotence have been reported in some male patients and resulted in discontinuance of tamoxifen therapy. Increased serum BUN and/or creatinine have also been reported in patients receiving tamoxifen as adjuvant therapy for breast cancer.

7. Musculoskeletal Effects
Musculoskeletal pain and bone pain have been reported in patients with breast cancer receiving tamoxifen. Increased bone and tumor pain or flare have occurred with tamoxifen therapy for metastatic breast cancer and are sometimes associated with a good tumor response. Patients with increased bone pain may require additional analgesics. Hypercalcemia, in some cases life-threatening, may occur during initial tamoxifen therapy in patients with metastatic breast cancer who have bone metastases. A potentially beneficial estrogenic effect of prolonged tamoxifen therapy is preservation of bone mineral density of the lumbar spine in postmenopausal women.

8. Ocular Effects
Tamoxifen rarely has been associated with ocular toxicity. Retinopathy, corneal opacities, and decreased visual acuity have occurred in patients receiving extremely high dosages (e.g., 180-320 mg daily) of tamoxifen for longer than 1 year. Ocular effects such as visual disturbances, decrement in color vision perception, corneal changes, cataracts, need for cataract surgery, optic neuritis, retinal vein thrombosis, intra-retinal crystals, posterior subcapsular opacities, and/or retinopathy have also been reported in patients receiving recommended dosages of the drug.

9. Hepatic Effects
Changes in hepatic enzyme concentrations (e.g., increased serum AST [SGOT] or ALT [SGPT] concentrations) and increased bilirubin and/or alkaline phosphatase concentrations have been reported in patients receiving tamoxifen therapy. Rarely, more severe hepatic abnormalities, including fatty changes in the liver, cholestasis, hepatitis, hepatic necrosis, and death, have occurred.

10. Gastrointestinal Effects
Adverse GI effects of tamoxifen, including nausea, anorexia, distaste for food, and abdominal cramps, have been reported in patients with breast cancer.

11. Nervous System Effects
Adverse nervous system effects reported in patients receiving tamoxifen for metastatic breast cancer include dizziness, lightheadedness, headache, fatigue, and mental depression.

12. Hematologic Effects
Thrombocytopenia (platelet counts of 50,000-100,000/mm3 and, infrequently, lower) occasionally has occurred in patients receiving tamoxifen for the treatment of breast cancer; however, platelet counts returned to normal even though tamoxifen therapy was continued. Hemorrhagic episodes have occurred rarely in patients with severe thrombocytopenia. Neutropenia, pancytopenia, and leukopenia (white blood cell count less than 3000/mm3), sometimes associated with anemia and/or thrombocytopenia, also have been reported and may be severe.

13. Dermatologic Effects
Thinning and/or partial loss of hair occurs infrequently in patients receiving tamoxifen for metastatic breast cancer. Erythema multiforme, Stevens-Johnson syndrome, and bullous pemphigoid have been reported rarely in patients receiving tamoxifen.

14. Other Adverse Effects
Other adverse effects reported in patients receiving tamoxifen as adjuvant therapy for breast cancer or for metastatic breast cancer include weight loss, fatigue, and

cough.

15. Geriatric Precautions

In the BCPT, 16% of the study participants were 65 years of age or older and 6% were at least 70 years of age. Reductions in the incidence of breast cancer were observed in women receiving tamoxifen across all age groups. Across all other outcomes, the results in the subset of women 65 years of age or older reflect the results observed in the subset of women at least 50 years of age. In this trial, the risk of serious adverse effects (e.g., endometrial cancer, pulmonary embolism, deep-vein thrombosis, stroke) was greatest in women 50 years of age and older.

16. Carcinogenicity

- Uterine Cancer

An increased incidence of uterine cancer, sometimes fatal, has been reported in women receiving tamoxifen. Most uterine malignancies associated with tamoxifen therapy are classified as adenocarcinoma of the endometrium; however, rare uterine sarcomas, including malignant mixed mullerian tumors, also have been reported. Uterine sarcoma has been reported more frequently among women receiving long-term therapy (i.e., exceeding 2 years) with tamoxifen than among women not receiving the drug. Among women enrolled in the BCPT, uterine sarcomas were reported in 4 women receiving tamoxifen versus none of the women receiving placebo (an incidence of 0.17 and 0 per 1000 women-years, respectively). Uterine sarcoma generally is associated with more advanced disease at the time of diagnosis, poorer prognosis, and shorter survival. After approximately 6.8 years of follow-up in the National Surgical Adjuvant Breast and Bowel Project (NSABP B-14) study, 15 of 1419 women randomized to receive tamoxifen 20 mg daily for 5 years developed uterine cancer; 2 of 1424 women who were receiving placebo initially, but who subsequently received tamoxifen for recurrent breast carcinoma, also developed uterine cancer. The relative risk of endometrial cancer in the tamoxifen-treated women was 7.5; most of the uterine cancers in tamoxifen-treated patients with breast cancer were diagnosed at an early stage, but deaths resulting from uterine cancer associated with tamoxifen therapy for the treatment of breast cancer have been reported. Endometrial hyperplasia can be a premalignant change. In one study of postmenopausal women receiving tamoxifen for the prevention of breast cancer, 16% developed atypical hyperplasia while no cases occurred in those receiving placebo; 8% of women receiving tamoxifen had an endometrial polyp compared with 2% of those receiving placebo. Endometriosis also has been reported. The manufacturer states that patients receiving or having previously received tamoxifen should undergo routine gynecologic examinations, and they should be advised to report promptly any menstrual irregularities, abnormal vaginal bleeding, change in vaginal discharge, or pelvic pain/pressure to their clinician; the cause of

such effects should be evaluated promptly.

- Liver Cancer
  In a study of women with breast cancer receiving tamoxifen (40 mg daily) or no adjuvant endocrine therapy for 2-5 years, 3 cases of liver cancer were reported in women receiving tamoxifen versus 1 case in the control group. No cases of liver cancer currently have been reported (at a median follow-up of 4.6 years) among women receiving either tamoxifen or placebo in the BCPT.

17. Pregnancy, Fertility, and Lactation
    - Pregnancy

      Tamoxifen may cause fetal harm when administered to pregnant women. Effects on reproductive function are expected from the antiestrogenic properties of the drug. Although the clinical importance of these changes is unknown, some of these changes, especially vaginal adenosis, are similar to those observed in young women who were exposed to DES in utero; such women have a greater risk (1 in 1000) of developing clear-cell adenocarcinoma of the vagina or cervix. To date, in utero exposure to tamoxifen has not been shown to cause vaginal adenosis or clear-cell adenocarcinoma of the vagina or cervix in young women. There are no adequate and well-controlled studies using tamoxifen in pregnant women. There have been reports of spontaneous abortions, birth defects, fetal deaths, and vaginal bleeding. Women should not become pregnant while receiving the drug, and those of childbearing potential should use an effective barrier or other nonhormonal method of contraception during tamoxifen therapy. When tamoxifen is administered during pregnancy or if the patient becomes pregnant while receiving the drug or within approximately 2 months after discontinuance of therapy with tamoxifen, the patient should be informed of the potential hazard to the fetus, including the possible long-term risk of a DES-like syndrome. For sexually active women of childbearing potential who are receiving tamoxifen for reduction in the incidence of breast cancer, therapy with the drug should be initiated during menstruation. In women with menstrual irregularity, pregnancy testing with a negative a-HCG should be confirmed immediately prior to the initiation of tamoxifen therapy.

    - Fertility
      A decreased number of implantations, as well as death of all fetuses, was observed in reproduction studies in male and female rats receiving tamoxifen.

    - Lactation
      It is not known if tamoxifen is distributed into milk. Because of the potential for serious adverse reactions to tamoxifen in nursing infants, a

decision should be made whether to discontinue nursing or the drug, taking into account the importance of the drug to the woman.

B. Bladder Biopsy

Biopsy of the bladder requires an anesthetic and there may be some bleeding afterwards.

C. Cystoscopy

The discomfort is nearly identical to being catheterized, which generally causes slight to moderate discomfort. There will be a feeling of fullness in the bladder and a sensation to empty during the cystoscopy examination. Infection or bladder irritation may also occur.

E. Confidentiality

The loss of confidentiality regarding research information is a possibility, although, the risk is extremely small. The investigator and his/her staff will make every effort to maintain the confidentiality.

Benefits:

Subject - The potential benefit to the subject is that tamoxifen may prevent or retard recurrence of the disease, and also the progression to invasive stages, ultimately prolonging survival. In our opinion, anticipated benefits outweigh the risks. While there is a small risk of thrombo-embolic phenomena and cardiovascular side effects (and other non-life-threatening adverse effects mentioned earlier), there is a significant chance that tamoxifen may be used successfully in patients with bladder cancer potentially reducing the burden, invasiveness, and costs of the disease.

Society - The potential benefit to society is that tamoxifen may retard the progression of bladder cancer and even shrink the tumors, improving survival and/or quality of life in these patients. New and non-toxic treatments for low/intermediate-risk bladder cancer are warranted since current therapies for this stage are unsatisfactory. Therefore, tamoxifen may fulfill an important role in the therapy armamentarium against bladder cancer. It offers the promise of efficacy with the ease of oral administration and lack of serious intravesical chemotherapy associated toxicities. The knowledge gained from this study will allow us to design next phase II and III

trials to address more definitive questions about oncological outcomes and survival with this new therapeutic strategy.

Risk to Benefit Ratio – In our opinion, anticipated benefits outweigh the risks. While there is a small risk of thromboembolic phenomena and cardiovascular side effects (and other non-life threatening adverse effects mentioned earlier in the potential discomforts section), there is an insignificant likelihood of toxicities associated with chemotherapy including neutropenic infections and gastrointestinal side effects.

Alternatives:
There is no alternative to participating in this study. Patients can decline participation or withdraw from the study voluntarily at any time and this will not affect whatsoever in any means their continuation of care or access to stand of care treatment for his condition.

Informed Consent Process:

Subjects will be identified from those visiting in the Scott Department of Urology (SDU) clinic, clinics of BCM-Affiliated Institutions, or Co-Investigators' sites. Patients will be informed both verbally and in written form of the study and procedures involved and be given adequate opportunity to read it and discuss with family before it is signed. The PI and/or the study coordinator will obtain a signed/dated Informed Consent Document (ICD) before enrolling each subject. The original signed/dated ICD will be kept with the research study documents, a copy will be given to the subject, and a copy will be placed in the subject's medical chart.

The BCM short form Spanish/English ICD versions will be submitted as an attachment in BRAIN and available for use if necessary.

Costs to Subjects:
The subjects or their health plan/insurance companies will need to pay for some or all of the costs of treating the cancer in this study. Some health plans will not pay these costs for people taking part in studies.

The study drug, tamoxifen, will be supplied to the subjects free of charge for the duration of the study. The costs associated with the second resection will be paid by the study.

The retention and submission of the samples are being done for research purposes. The subjects or their insurance companies will not be billed for the preparation of these samples or the testing that will be done with these samples. These test results will not be made part of the subjects' medical records as they are for research purposes only.

Payment to Subjects:
Subjects will not be paid for taking part in this study.

Confidentiality:

Research data and regulatory documents will be managed by Research Administration of the Scott Department of Urology. There will be three actual physical locations, the department's Faculty Center suites 1250 and 1650, and the other is Main Baylor, Jewish Wing, offices 502D and 506D. The Faculty Center suite utilizes an electronic locking system for security purposes. The Main Baylor offices have keyed entries. All computers are password protected.

The Scott Department of Urology complies fully with the HIPAA Privacy Rule.

The BCM Dan L. Duncan Cancer Center (DLDCC), Harris County Hospital District - Ben Taub General Hospital (BTGH), St. Luke's Episcopal Hospital (SLEH), and The Methodist Hospital Research Institute (TMHRI) will have access to patient information related to this study.

Data and Safety Monitoring Plan

This Investigator-Initialed study will utilize the Baylor College of Medicine Dan L. Duncan Cancer Center's Data Review Committee (DRC) as a resource for data and safety monitoring. The DLDCC DRC will perform data monitoring on a regular basis. Clinical safety data including the following will be evaluated:

- Overall protocol accrual and expected number of patients to be treated
- Patient registrations with regard to eligibility and evaluability
- All adverse events and their relationship to the protocol therapy (e.g., by dose level, treatment arm, etc)
- All serious adverse events requiring expedited reporting as defined in the protocol
- Whether participants are being exposed to unanticipated or excessive toxicity
- Results of any planned interim analyses
- Response evaluations, if relevant
- Whether protocol specific rules are being followed
- Status of participation rate of correlative biology and/or imaging studies, if applicable
- Study amendments/modifications that may have occurred since the last review
- All annual study data

Adverse Events

The AE collection/reporting period will begin with the first day of treatment with Tamoxifen. AEs after study registration but prior to the first day of study treatment will be captured as ongoing concurrent secondary diagnoses and symptoms present at the start of study. AEs will be graded in accordance with the NCI Common Terminology Criteria for Adverse Events v4.03 (CTCAE) http://ctep.cancer.gov/reporting/ctc.html and will be reported in accordance to all current applicable FD< ICH and IRB regulation and guidelines.

An attribution scale which will be utilized to attribute relatedness of toxicity to the study. The following scale may be used:

- Definite – The AE is clearly related to the study treatment.
- Probable – The AE is likely related to the study treatment.
- Possible – The AE may be related to the study treatment.
- Unlikely – The AE is doubtfully related to the study treatment.
- Unrelated – The AE is clearly NOT related to the study treatment.

A definition of expected adverse event and unexpected adverse event.

Expected adverse events are those that have been previously identified as resulting from administration of the agent. For purposes of this study, an adverse event is considered expected when it appears in the current adverse event list in the Investigator's Brochure, the package insert, or is included in the informed consent document as a potential risk.

An adverse event is considered unexpected when it varies in nature, intensity, or frequency from information provided in the current adverse event list in the Investigator's Brochure, the package insert or when it is not included in the informed consent document as a potential risk.

Adverse Event Reporting

All adverse events, regardless of perceived relationship to study treatment, will be reported and recorded on the appropriate CRFs. New AEs and SAEs that are ongoing at the end of the study will be followed for 30 days from the patient's receipt of the last dose of protocol therapy, unless they have resolved earlier. SAEs and drug related AEs ongoing at the end of study will be followed until resolution.

The AE description will include the nature of the experience, the date of onset, the resolution date, the severity of each sign or symptom reported using the NCI-CTCAE (version v4.03), the seriousness of the event, the potential relationship to study treatment, the course of action taken, and the outcome of the experience.

Serious adverse events are to be reported to the institutional review board (IRB) according to each board's reporting requirements and required time frame.

The Study Chair/Principal Investigator will be responsible for reporting all adverse events to the FDA as per their reporting requirements and time frame.

Pregnancy

This study will exclude women of child bearing potential. However, the following procedures will be followed in the unlikely event of a pregnancy on study. A subject who has a positive βhCG pregnancy test result at any time after the first dose of investigational therapy will be immediately withdrawn from participation in the study. All study conclusion/withdrawal assessments will be collected at the time of discontinuation. Pregnancy information is to be recorded in the Case Report Forms. Pregnancy information will be collected from the first dose of investigational product to 30 days after the last dose. While pregnancy itself is not considered to be an SAE, it may result in an SAE. Any pregnancy complication or elective termination of a pregnancy for medical reasons will be recorded as an SAE and will be reported as such. A spontaneous abortion is always considered an SAE and will be reported as such.
The AE collection/reporting period will begin with the first day of treatment with Tamoxifen. AEs after study consenting but prior to the first day of study treatment will be captured as ongoing concurrent secondary diagnoses and symptoms present at the start of study. AEs will be graded in accordance with the NCI Common Terminology Criteria for Adverse Events Adverse Events will be reported in accordance to all current applicable FDA, ICH, and IRB rules, regulations, and guidelines.

Data Handling and Record Keeping

Case Report Forms

Protocol-specific data will be collected on Case Report Forms as required and forwarded to BCM for compilation by the data manager. The completed dataset is available to the PI, is the sole property of BCM, and should not be made available in any form to third parties, except for authorized representatives of appropriate Health/Regulatory Authorities, without written permission from BCM.

Record Retention

To enable evaluations and/or audits from Health Authorities/BCM, the investigator agrees to keep records, including the identity of all participating patients (sufficient information to link records; e.g., hospital records), all original signed informed consent forms, copies of all CRF's, and detailed records of drug disposition. To comply with international regulations, the records should be retained by the investigator in compliance with regulations.

During data entry, range and missing data checks will be performed on-line. The checks to be performed will be documented in the Data Monitoring Plan for the study. A summary report (QC Report) of these checks together with any queries resulting from manual review of the CRF's will be generated for each site and transmitted to the site and the site monitor. Corrections will be made by the study site personnel. This will be done on an ongoing basis**.**

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Appendices

Appendix I Tamoxifen Package Insert

PACKAGE INSERT TEMPLATE FOR TAMOXIFEN TABLET
Brand or Product Name
[Product name] Tablet 10mg
[Product name] Tablet 20mg
Name and Strength of Active Substance(s)
Tamoxifen citrate … …equivalent to tamoxifen....mg

Product Description

[Visual description of the appearance of the product (eg colour, markings etc)
eg White, circular flat beveled edge film-coated dispersible tablets marked '20' on one side

Pharmacodynamics

Tamoxifen is the trans-isomer of a triphenylethylene derivative. It is a nonsteroidal antiestrogen which that competes with estrogen for estrogen receptor positive on breast cancer cells thereby preventing their growth. It displays a complex spectrum of oestrogen antagonist and oestrogen agonist-like pharmacological effects in different tissues. For example, tamoxifen acts as an estrogen receptor antagonist in the breast, but as an agonist in the uterus. Tamoxifen has been shown to up-regulate both estrogen and progesterone receptors in breast cancer.

Tamoxifen's estrogen-like influence on the skeletal and cardiovascular systems reduces postmenopausal bone loss and produces favorable cholesterol, lipid, and lipoprotein profiles.

Tamoxifen's anticancer activity was originally thought to be due solely to its ability to compete with estrogen for binding sites (estrogen receptors, ER) in target tissues such as the breast. However, other recognized actions include inhibition of protein kinase C and Ca(2+)-calmodulin-dependent cAMP phosphodiesterase, induction of cells surrounding the cancer cells to secrete the negative growth factor transforming growth factor-beta (TGF-beta), and suppression of insulin-like growth factor I (IGF-1), which is a potent mitogen for breast cancer cell in vitro .

Pharmacokinetics

Absorption

Tamoxifen is well absorbed from the gastrointestinal tract.

Peak plasma concentrations of tamoxifen occur 4 to 7 hours after an oral dose.

Distribution

It is extensively protein bound *Updated November 2012* 2

Metabolism

It is extensively metabolised by the cytochrome P450 isoenzymes CYP3A4, CYP2C9, and CYP2D6. The major serum metabolite N-desmethyltamoxifen(active) which is formed mainly via CYP3A4; 4-Hydroxytamoxifen is a minor metabolite. N-Desmethyltamoxifen and 4-hydroxytamoxifen are further metabolised (via CYP2D6 and the CYP3A family, respectively) to 4-hydroxy-N-desmethyltamoxifen (endoxifen). Several of the metabolites are stated to have similar pharmacological activity to the parent compound, and endoxifen has been proposed as an alternative treatment to avoid differences in metabolism.

Elimination

Plasma clearance is reported to be biphasic and the terminal half-life may be up to 7 days. Tamoxifen is excreted slowly in the faeces, mainly as conjugates. Small amounts are excreted in urine. Tamoxifen appears to undergo enterohepatic circulation

N-desmethyltamoxifen has a half-life at steady state of about 14 days.

Indication

Tamoxifen is indicated for the treatment of breast cancer.

Recommended Dosage

Adults (including elderly): The dosage range is 20 to 40 mg daily, given either in divided doses twice daily or as a single dose once daily.

Use in children

The use of tamoxifen is not recommended in children, as safety and efficacy have not been established

Mode of Administration

Oral

Contraindications

Tamoxifen must not be given during pregnancy. Premenopausal patients must be carefully examined before treatment to exclude the possibility of pregnancy
Hypersensitivity to the product or any of its ingredients When used for the reduction in breast cancer incidence in high risk women and women with Ductal Carcinoma in Situ with *Updated November 2012* 3

- concomitant coumarin-type anticoagulant therapy
- history of deep vein thrombosis
- history of pulmonary embolus

Warnings and Precautions

- Menstruation is suppressed in a proportion of premenopausal women receiving tamoxifen for the treatment of breast cancer.
- Uterine malignancies; risk of potentially fatal uterine sarcoma and endometrial carcinoma; risk increases with long-term use
- Endometrial changes including hyperplasia and polyps; increased risk

*Any patient receiving or having previously received Tamoxifen, who report abnormal gynaecological symptoms, especially vaginal bleeding, or who presents with menstrual irregularities, vaginal discharge and symptoms such as pelvic pain or pressure should be promptly investigated.

- fibroids, uterine, endometriosis; increased risk
- liver abnormalities; fatty liver, cholestasis, hepatitis, and hepatic necrosis, some fatal, have been reported
- liver enzyme levels; changes have been observed
- metastatic breast cancer; hypercalcemia has been reported in some patients with bone metastases
- ocular disturbances including cataracts (some requiring surgery), corneal changes, decrease in color vision perception, retinal vein thrombosis, and retinopathy; increased risk
- premenopausal patients with advanced breast cancer; increased risk of ovarian cysts

- Prescribers should obtain careful histories with respect to the patient's personal and family history of Venous thromboembolism. If suggestive of a prothrombotic risk, patients should be screened for thrombophilic factors. Patients who test positive should be counselled regarding their thrombotic risk. The decision to use tamoxifen in these patients should be based on the overall risk to the patient. In selected patients, the use of tamoxifen with prophylactic anticoagulation may be justified

- thromboembolic events, including stroke, deep vein thrombosis, and pulmonary embolism; increased risk

- Increased risk for Venous thromboembolism (VTE) has been demonstrated in healthy tamoxifen-treated women .The risk of VTE is further increased by severe obesity, increasing age and all other risk factors for VTE. The risks and benefits should be
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carefully considered for *all* patients before treatment with tamoxifen. This risk is also increased by concomitant chemotherapy . Long-term anticoagulant prophylaxis may be justified for some patients who have multiple risk factors for VTE.

 Surgery and immobility: Tamoxifen treatment should only be stopped if the risk of tamoxifen-induced thrombosis clearly outweighs the risks associated with interrupting treatment. All patients should receive appropriate thrombosis prophylactic measures and should include graduated compression stockings for the period of hospitalisation, early ambulation, if possible, and anticoagulant treatment.

 If any patient presents with VTE, tamoxifen should be stopped immediately and appropriate anti-thrombosis measures initiated. The decision to re-start tamoxifen should be made with respect to the overall risk for the patient. In selected patients, the continued use of tamoxifen with prophylactic anticoagulation may be justified.

Effects on the ability to drive and use machines
There is no evidence that tamoxifen results in impairment of these activities

Interactions with Other Medicaments

 There is a risk of increased anticoagulant effect if tamoxifen is given with coumarin anticoagulants. Conversely, use with cytotoxic drugs may increase the risk of thromboembolic events; prophylactic anticoagulation should be considered. In order to avoid bleeding during a possible thrombocytopenic episode, platelet aggregation inhibitors should not be used with tamoxifen.

 Tamoxifen increases the dopaminergic effect of bromocriptine; bromocriptine increases serum concentrations of tamoxifen and its major metabolite N-desmethyltamoxifen. Use with inhibitors of cytochrome P450 isoenzyme CYP2D6 has been shown to reduce plasma concentrations of endoxifen, a tamoxifen metabolite; while the clinical relevance is unclear, a reduced effect of tamoxifen cannot be excluded, and use with potent CYP2D6 inhibitors should be avoided if possible.

 Tamoxifen is metabolised by CYP3A4 and care is required when it is used with CYP3A4 inducers (such as rifampicin), as tamoxifen concentrations may be reduced; plasma concentrations of tamoxifen may be increased by use with CYP3A4 inhibitors.

 Preparations of sex hormones, especially oestrogens, should not be used with tamoxifen as a mutual decrease in effect is possible. Medroxyprogesterone decreases concentrations of N-desmethyltamoxifen, but not of tamoxifen.

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 Tamoxifen is metabolised by cytochrome P450 isoenzymes, including CYP2D6 , and drugs which inhibit CYP2D6 can reduce metabolism and potentially increase the risk of breast cancer recurrence.

 Potent inhibitors should be avoided; these include the SSRIs paroxetine, fluoxetine, bupropion, and duloxetine, and other drugs such as perphenazine, pimozide, thioridazine, quinidine, ticlopidine, terbinafine, and cinacalcet.

 Weak to moderate inhibitors should be used with caution, especially in patients considered to be CYP2D6 intermediate metabolisers. These include the SSRIs sertraline, citalopram, and fluvoxamine, the tricyclic antidepressants clomipramine, doxepin, desipramine, imipramine, amitriptyline, and nortriptyline, as well as other drugs such as chlorpromazine, fluphenazine, haloperidol, amiodarone, amlodipine, felodipine, nicardipine, nifedipine, verapamil, chloroquine, halofantrine, ritonavir, cimetidine, clemastine, diphenylpyraline, hydroxyzine, promethazine, tripelennamine, and celecoxib

The use of tamoxifen in combination with an aromatase inhibitor as adjuvant therapy has not shown improved efficacy compared with tamoxifen alone. (Innovator)
Concurrent use of following drugs and tamoxifen may result in decreased plasma concentrations of the active metabolites of tamoxifen

- cimetidine
- quinidine
- diphenhydramine,
- mifepristone
- thioridazine

Concurrent use of St John's Wort and tamoxifen may result in reduced tamoxifen effectiveness.
Concurrent use of tamoxifen and mitomycin may result in increased risk of hemolytic uremic syndrome.
Concurrent use of letrozole and tamoxifen may result in reduced letrozole serum concentrations.
Concurrent use of clobazam and tamoxifen may result in increased tamoxifen plasma concentrations.
Concurrent use of anastrozole and tamoxifen may result in reduced anastrozole plasma levels.
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Concurrent use of clopidogrel and tamoxifen may result in an increased risk of tamoxifen toxicity (nausea, vomiting, dizziness, hyperreflexia, qt prolongation, increase in liver function tests).

Statement on Usage During Pregnancy and Lactation

Pregnancy

Although there are no adequate and well-controlled studies of tamoxifen use in pregnant women, there have been a few reports of spontaneous abortions, birth defects, fetal deaths, and vaginal bleeding associated with tamoxifen use during pregnancy. Therefore, if tamoxifen is administered during pregnancy or if the patient becomes pregnant while receiving tamoxifen or within 2 months after therapy discontinuation, the woman should be informed of the potential risks to the foetus . Women should be advised not to become pregnant whilst taking tamoxifen and should use barrier or other non-hormonal contraceptive methods if sexually active. For women who are sexually active and of childbearing potential initiate tamoxifen during menstruation. If menstruation is irregular, a negative pregnancy test (beta-hCG) immediately before starting tamoxifen treatment should be adequate.

Lactation

It is not known whether tamoxifen is excreted into human breast milk and the potential for adverse effects in the nursing infant from exposure to the drug are unknown. Tamoxifen has been shown to significantly inhibit postpartum lactation. Because tamoxifen exposure has the potential to cause harm in a nursing infant, nursing mothers who are on tamoxifen therapy should not breastfeed. The decision either to discontinue nursing or discontinue tamoxifen should take into account the importance of the drug to the mother.

Adverse Effects / Undesirable Effects

When side effects are severe, it may be possible to control them by a simple reduction of dosage (to not less than 20 mg/day) without loss of control of the disease. If side effects do not respond to this measure, it may be necessary to stop the treatment.
Dermatologic: Menopausal flushing, skin rashes (including rare reports of erythema multiforme, Stevens-Johnson syndrome, cutaneous vasculitis, and bullous pemphigoid) dry skin and alopecia,
Reproductive: Irregular periods, vaginal discharge vaginal bleeding pruritus vulvae, uterine cancer, uterine fibroids and endometrial changes including hyperplasia and polyps may occur, and an increased incidence of endometrial carcinoma, and rarely uterine sarcoma, has been reported. Suppression of

menstruation may occur in premenopausal women and cystic ovarian swellings have occasionally occurred. *Updated November 2012* 7

Endocrine metabolic: Breast cancer, contralateral
Hepatic: Tamoxifen has been associated with increased liver enzymes, and rarely with cholestasis and hepatitis.
Gastrointestinal: nausea, gastrointestinal intolerance
Neurologic: dizziness, headache, depression, confusion
Haematologic: Thromboembolic disorder/events; deep venous thrombosis. Transient thrombocytopenia and leucopenia have been reported
*When Tamoxifen is used in combination with cytotoxic agents, there is an increased risk of thromboembolic events occurring.
Ophthalmic: Cataract, visual disturbances (blurred vision and loss of visual acuity, corneal opacities, retinopathies, and cataracts have occurred rarely)
*Cases of optic neuropathy and optic neuritis have been reported in patients receiving tamoxifen and, in a small number of cases, blindness has occurred
Respiratory: Interstitial pneumonitis, pulmonary embolism
Other: Breast cancer; receptor-negative, uterine cancer, fatigue, hypertriglyceridaemia in some cases with pancreatitis, fluid retention, muscle cramps, hypersensitivity reactions, including angioedema.Tumour pain and flare may be a sign of response, but hypercalcaemia, sometimes severe, has developed in patients with bony metastases.

Overdose and Treatment

Symptoms

Overdoses are extremely rare; however, as they are widely used, adverse effects are common. There is very little information regarding overdose of these compounds in humans. At doses 6 times (400 mg/m(2) the recommended doses (20 to 40 mg daily), neurotoxicity (seizures, tremor, hyperreflexia, unsteady gait, and dizziness) and ECG changes (prolonged QT interval) were noted.

Treatment

There is no specific antidote to overdosage and treatment must be symptomatic. *Updated November 2012* 8

Storage Conditions

[eg Store below….□ C]

Dosage Forms and Packaging Available

[Packaging type & pack size]

Name and Address of Manufacturer

[Name & full address of manufacturer]

Name and Address of Marketing Authorization Holder

[Name & full address of marketing authorization holder]

Date of Revision of Package Insert

[day/month/year]