THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center

Protocol Page

A Phase II Study of Preoperative Capecitabine and Concomitant Radiation in Women with Advanced Inflammatory or Non-Inflammatory Breast Cancer 2009-0087

Core Protocol Information

Short Title	Xeloda and external beam radiation
Study Chair:	Wendy A. Woodward
Additional Contact:	Beth DeGracia Donna M. White Toni Williams
Department:	Radiation Oncology
Phone:	713-794-4892
Unit:	1202
Full Title:	A Phase II Study of Preoperative Capecitabine and Concomitant Radiation in Women with Advanced Inflammatory or Non-Inflammatory Breast Cancer
Protocol Type:	Standard Protocol
Protocol Phase:	Phase II
Version Status:	Terminated 06/22/2017
Version:	08
Submitted by:	Donna M. White--11/2/2012 2:52:51 PM
OPR Action:	Accepted by: Matthew D. Geidel -- 11/14/2012 2:48:38 PM

Which Committee will review this protocol?

◉ The Clinical Research Committee - (CRC)

Protocol Body

1.0 Objectives

Primary

The primary objective of the study is to determine the rate of response by RECIST criteria (Appendix F) in all patients who receive treatment. This includes the following:

- Pre-operative or palliative concurrent radiation with capecitabine to the breast and at risk or involved regional lymph node basins

i. Efficacy

The secondary efficacy objectives of the study are:

- To determine the rate of conversion to operable, cCR, and pCR after completion of all protocol specified therapy. This includes the following:
  - Pre-operative concurrent radiation with capecitabine to the breast and at risk or involved regional lymph node basins
- To determine locoregional control of unresected nodal disease treated to definitive radiation dose ($\geq$ 60Gy)

ii. Safety

The secondary safety objectives of the study are:

- Determine the rate of post-surgical wound complications after pre-operative radiation and capecitabine among patients who undergo surgery
- Determine the rate of grade III toxicities (excluding acute skin toxicity)

iii. Biomarker Research

The biomarker research objectives of the study are:

- To determine if change in the absolute number of circulating tumor cells (CTCs) in the blood during pre-operative radiation with chemotherapy predicts for percentage of viable cells in a tumor core biopsy expressing putative stem/progenitor cell markers. (Patients whose gross disease cannot be completely encompassed by a combination of locoregional therapy with chemo/HOU/UTMDACCiation and surgery will be analyzed separately).
- To identify tumor and serum biomarkers present at baseline that could be predictive of cCR or benefit of radiation. These will be examined in IBC vs. non-IBC samples.

2.0 Rationale

Locally advanced breast cancer can be painful and distressing at any time during a patients' breast cancer treatment course. Palliative or pre-operative radiation for patients with non-metastatic disease and selected patients with metastatic disease has been used in reduce or eliminate disease, provide symptom control, and convert inoperable cancers to operable. For example, in patients who present with non-metastatic disease, progressive disease or minimal response after neoadjuvant chemotherapy is a poor prognostic factor even when definitive surgery can be performed, and this cohort contains patients who are not eligible for surgery based on the size of the primary. Alternatively some patients will develop progressive or inoperable breast cancer as a recurrence after definitive therapy for breast cancer, and finally patients who present or recur with distant disease are often not surgical candidates but may benefit from palliative radiation to the primary tumor, or definitive radiation in select patients with limited or controlled metastatic disease. We propose a prospective single arm trial of radiation and capecitabine, a radiosensitizer, to improve response rates in these patients, for whom currently there are limited therapeutic options.

Significant experience using pre-operative radiation and Capecitabine for rectal cancer have led to preliminary MDACC experience using concurrent pre-operative Capecitabine and radiation for inoperable breast cancer (GHP, unpublished data), and a small published phase II trial (N = 28) supports this approach for breast cancer patients resistant to first line chemotherapy to improve operability1. Here we propose to prospectively examine the efficacy of this approach in terms of response in a larger, clinically broad group of IBC and non-IBC patients for whom pre-operative radiation is offered as the standard of care. Secondary analysis comparing these groups will be used to generate hypotheses regarding treatment approaches for IBC patients.

The pre-operative radiation approach offers the opportunity to examine important translational questions regarding biological markers and surrogates of response using this short –term response endpoint as well as tumor tissue quantitation of tumor stem cells. An important translational endpoint of this trial will be to evaluate circulating tumor cells (CTCs) as surrogate cancer stem cell markers and to correlate the change in tumor stem cell markers after therapy to change in CTCs. As such if the trial has not met early stopping rules at total accrual for the primary endpoint and insufficient patients have consented to the translational component of the trial to provide adequate statistical power to address this translational endpoint, we will plan to request a protocol amendment to increase accrual based on statistical power necessary to achieve the secondary translational endpoint.

3.0 Background

3.1 Increasing need for effective palliative and pre-operative local therapy for in situ gross disease

Inflammatory breast carcinoma (IBC) is one of the most aggressive forms of primary

breast carcinoma that accounts for 1-6% of all invasive breast tumors in the United States and Western Europe2. IBC is distinguished from other types of breast cancer by clinical, pathologic and molecular features and is classified by the American Joint Committee on Cancer (AJCC) as a separate clinicopathological entity (T4d).
Opening of a dedicated IBC clinic has dramatically increased the number of IBC patients, and due to the natural history of this disease, a commiserate increase in patients with in situ disease resistant to chemotherapy for whom palliative or pre-operative radiation is clinically appropriate.

3.2 Pre-operative breast radiation alone

Treatment of non-metastatic/oligometastatic IBC and locally advanced non-IBC consists of neoadjuvant chemotherapy, mastectomy and axillary lymph node dissection, followed by adjuvant radiation. In patients with progressive disease during neoadjuvant chemotherapy, widespread metastatic disease at presentation, and inoperable disease either at presentation or after neoadjuvant chemotherapy, or gross in breast/chest wall recurrence, pre-operative radiation is a component of appropriate standard of care to achieve tumor and symptom control and potentially render patients operable. Local control in 42 IBC patients treated at MDACC with pre-operative or definitive radiation for gross disease (40/42 without concurrent chemotherapy) was 75% at 5 years. Distant metastasis free survival was 20% with 8 patients alive without evidence of distant metastasis at $\geq$ 40 months of follow up (range among women alive without evidence of disease 40 – 240 months, unpublished data).

Among 38 non-IBC patients treated with radiation for inoperable breast cancer resistant to anthracycline-containing primary chemotherapy on five consecutive MDACC institutional trials without evidence of distant metastases at diagnosis revealed thirty-two (84%) of the 38 patients were able to undergo mastectomy after radiotherapy 3. For the whole group, the overall survival rate at 5 years was 46%, with a distant disease-free survival rate of 32%. The 5-year survival rate for patients who were inoperable because of primary disease extent was 64% compared with 30% for those who were inoperable because of nodal disease extent ($p = 0.0266$). The 5-year rate of locoregional control was 73% for the surgically treated patients and 64% for the overall group. Of the 32 who underwent mastectomy, the 5-year rate of significant postoperative complications was 53%, with 4 (13%) requiring subsequent hospitalization and additional surgical revision. Preoperative radiation doses of >or=54 Gy were significantly associated with the development of complications requiring surgical treatment (70% vs. 9% for doses <54 Gy, $p = 0.0257$) 3.

3.3 Capecitabine

Capecitabine (Xeloda®) is a fluoropyrimidine carbamate with documented antineoplastic activity approved as a first line agent for the treatment of metastatic breast cancer resistant to antracycline and taxane therapy. It is an orally administered systemic prodrug which is converted to 5-FU. It is readily absorbed from the GI tract and then in the liver

is hydrolyzed by hepatic carboxyl esterases (HCE) to 5-DFCR which is the first step of a triple enzyme pathway (TEP). 5-DFCR is converted by cytidine deaminase to 5-DFUR (enzyme two), and finally 5-DFUR is hydrolyzed by thymidine phosphorylase (TP) to the active drug 5-FU. Pharmacokinetics have been extensively studies and are included as an attachment to this protocol and available at http://www.rocheusa.com/products/xeloda/pi.pdf.

Capecitabine undergoes sequential conversion via the triple enzyme pathway to 5-FU. The final enzyme in the pathway is TP which is preferentially expressed in tumor cells as opposed to normal tissue cells thereby increasing the therapeutic index 4. TP is also upregulated in expression by external beam radiation therapy 5and therefore capecitabine potentially can have uniquely increased antitumor activity as well as improved therapeutic index when used concurrently with radiation (reviewed in 6).

3.4 Concurrent Capecitabine/HOU/UTMDACCiation in Non-breast Cancer

Capecitabine has undergone investigations in the setting of concurrent radiation therapy. Radiation therapy has been demonstrated to cause an increase in thymidine phosphorylase levels (TP) which is a rate limiting enzyme in the capecitabine pathway. The combination of radiation therapy and capecitabine therefore offers an opportunity to exploit synergy in order to increase tumoricidal activity.

Two Phase I dose-finding studies in rectal cancer investigated the feasibility of using concurrent radiotherapy and capecitabine and defined the maximum tolerated dose (MTD) (revewied in 7) In one study, dose-limiting toxicity (Grade 3 hand–foot syndrome) occurred at a capecitabine dose of 1000 mg/m2 BID, and a dose of 825 mg/m2 BID was recommended for further evaluation. Based on tumor imaging, 9 of 10 patients experienced a clinical PR 8. The MTD of capecitabine was also found to be 1000 mg/m2 BID in a rectal cancer study by Ngan et al in which 5 patients (19%) achieved a CR. As a result of these studies, the recommended dose of capecitabine with radiotherapy is 825 mg/m2 BID administered from the first to the last day of standard pelvic radiotherapy 9.

In the subsequent phase II rectal cancer study by Dunst et al, after a median follow-up of 48 months, the 5-year overall survival and tumor control data were, with regard to patient selection, in the expected range with an overall survival of 65%, a relapse-free survival of 47%, and a local recurrence rate after 5 years of 17%. The data confirmed that capecitabine is an adequate substitute for 5-fluorouracil in preoperative chemoradiation of rectal cancer with a favorable safety profile 10.

At The University of Texas M.D. Anderson, Krishnan et al conducted a phase II study of capecitabine (825 mg/m2 orally, twice daily continuous) with radiotherapy (52.5 Gy/30 fractions to the primary tumor and perirectal nodes) in 54 patients with locally advanced rectal cancer (node-negative > or = T3 or any node-positive tumor) staged by endoscopic ultrasound (EUS). The primary endpoint was pathologic response rate; secondary endpoints included toxicity profiles and survival parameters. Of the 54 patients (median

age, 56.7 years; range, 21.3-78.7 years; male:female ratio, 1.7; Eastern Cooperative Oncology Group performance status 0-1: 100%), 51 patients (94%) had T3N0 or T3N1 disease by EUS. Surgery was not performed in 3 patients; 2 of these patients had metastatic disease, and the third patient refused after a complete clinical response. Of the 51 patients evaluable for pathologic response, 9 patients (18%) achieved complete response, and 12 patients (24%) had microscopic residual disease (< 10% viable cells). In addition, 26 patients of all 54 patients (51%) achieved T-downstaging, and 15 patients of 29 patients (52%) achieved N-downstaging. Grade 3/4 toxicities were radiation dermatitis (9%) and diarrhea (2%). Sphincter preservation rate for tumor < or = 5 cm from the anal verge was 67% (18/27). This regimen of radiotherapy plus capecitabine is well tolerated and is more convenient than protracted venous infusion of 5-FU. The pathologic response rate is comparable to previous experience using protracted venous infusion 5-FU 11.

3.5 Pre-operative breast irradiation with concurrent capecitabine

Based on the long history of 5-FU chemotherapy for breast cancer and the encouraging safety and efficacy results reviewed above for pre-operative concurrent Capecitabine and radiation in rectal cancer, this approach was advocated for carefully selected breast cancer patients with inoperable breast cancer and no better treatment options at MDACC. In retrospective review of 55 patients treated with concurrent radiation and capecitabine at MDACC for inoperable breast cancer (IBC and non-IBC), concurrent chemoradiation with capecitabine demonstrated 91% of these patients converted to operable. The clinical complete response rate was 33%; moreover, the overall pathological CR rate was 20%. Only 1 patient had progressive disease. The 5-year OS, LRFFS, and DMFS rates were 48%, 85%, and 37%. Sixteen patients (29%) had a grade 3 or higher complication (acute yet resolving skin toxicity; (GHP, unpublished data).

In a phase II study of radiation and capecitabine in women with locally advanced breast cancer in patients who have failed first line anthracycline-based neoadjuvant therapy Gaui et al studied the concomitant use of radiation therapy and capecitabine in this setting, to determine the toxicity and efficacy of this regimen as a second-line neoadjuvant treatment 1. Twenty-eight patients with inoperable locally advanced breast cancer refractory to first-line anthracycline based treatment were enrolled between January 2003 and May 2004. Patients received radiation therapy (total dose 5000 cGy) and concomitant capecitabine (850 mg/m2) twice daily for 14 days every 3 weeks. This treatment rendered 23 of the 28 patients (82%) operable. The 5 remaining patients did not undergo surgery because of disease progression. The median clinical tumor size decreased from 80 cm2 to 49 cm2. Microscopic residual disease was observed in 3 patients (13%) and another patient achieved a complete pathologic response. The median number of involved lymph nodes was 2 and treatment was well tolerated with no grade 3 or 4 events. These results indicate that second-line neoadjuvant treatment with radiation therapy and capecitabine is feasible, well tolerated, and effective in patients with locally advanced breast cancer refractory to primary anthracycline-based treatment.

While these results are encouraging, prospective data are limited to the 28 patient study by Gaui et al 1. No prospective data have been collected for IBC patients, and both the response and complication data are difficult to fully assess retrospectively.

4.0 Protocol Specified Treatment

4.1 Dose Rationale for Concurrent Capecitabine and Radiation

Protocol participation is such that the treatment will be administered as an outpatient. Inpatient status is not required and change in status does not necessitate removal from protocol. Based on concurrent capecitabine radiation data in non-breast sites reviewed above as well as from our preliminary experienced reviewed retrospectively in pre-operative concurrent breast cancer, the dosage administered to each patient will be 825 mg/m2 bid. One of the two daily doses of capecitabine should be taken approximately 2 hours before receiving radiotherapy. The first day of Capecitabine is the the same day that radiotherapy is started and the last day that Capecitabine is given is the last day of radiotherapy. Capecitabine will be administered only on the days the patient receives radiation therapy.

Doses for capecitabine will be calculated on the basis of milligrams of drug per square meter of body surface area (BSA) as measured at baseline (mg/m2). The weight of the patient may change throughout the study, however, body surface area will be assumed to stay close to that measured at baseline and therefore will be the basis for all dose calculations during the study participation by the patient. The calculation will be performed using the UTMDACC clinicportal BSA calculator12. Once this value is obtained the result will be in mg/m2 and then will be rounded to the nearest number of whole 500 and 150 mg tablets so that two daily oral doses are given. The doses should be separated by 12 hours and are to be taken within 30 minutes after ingestion of food. The tablets should not be manually divided as they are not scored.

Protocol based radiation therapy dose will be 50-57 Gy to the initial clinical target volume (CTV, gross disease + tissue at risk for micrometastatic disease including margin around gross disease and draining regional lymphatics). Additional "boost" dose to bring the total dose to 60-72 Gy to gross target volumes (GTV) defined by the presence of gross disease on pre-treatment imaging is acceptable but not mandated by the protocol.

Optional Preoperative/definitive regimens include:
Any palliative regimen <3 Gy per fraction is allowable

1. 50-54 Gy at 2 Gy per fraction (one daily treatment) followed by optional GTV boost at 2 Gy per fraction to total dose;
2. 57 Gy at 1.8 Gy per fraction (IMRT) with optional nested GTV dose to total not to exceed 72 Gy at 2.2 Gy per fraction (Example Figure 1), or
3. 51 Gy at 1.5 Gy per fraction twice daily (bid) to the CTV followed by optional GTV boost at 1.5 Gy bid to total dose.

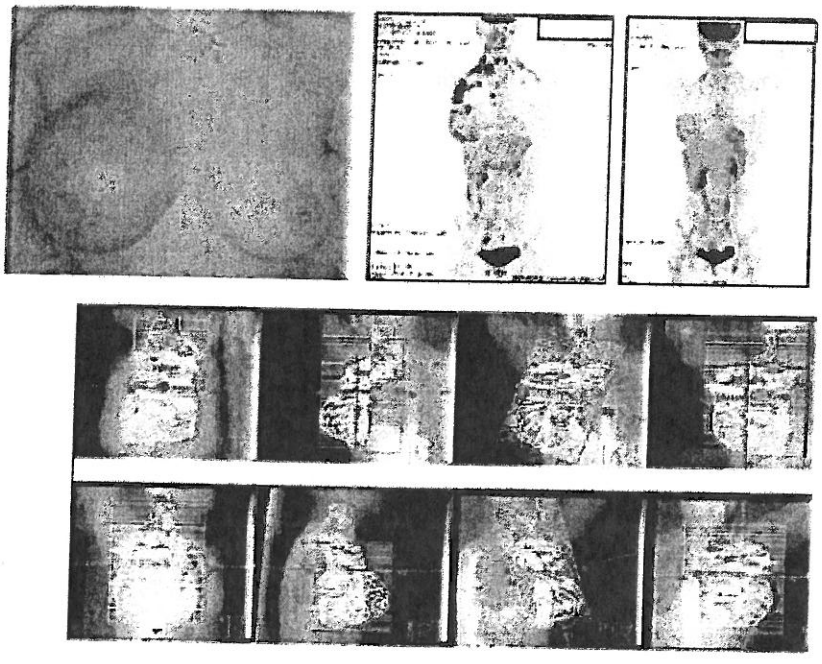


Figure 1. IBC patient (gross disease upper left, pre/post treatment PET upper right) treated with IMRT+ Xeloda to 57 1.8 to the CTV (lower panel, treatment plan yellow). 69.95 1.8 Gy to gross disease (red). Patient had margin negative surgery of breast and level I/II axilla without complication. Control of gross disease treated to 69.95 at 5 months.

Dose escalation ≥ 60 Gy to achieve local control of gross disease that will not be resected (patient remains inoperable in surgical consultation prior to completing radiation treatment. patient has gross disease in sites not resected in standard surgery such as IMC nodes and supraclavicular nodes) will be at the discretion of the treating physician (See example. Figure 1)

Guidelines for GTV dose escalation:

- 60-66Gy to gross disease <1 cm, up to 72 Gy to gross disease ≥ 1 cm when standard normal tissue constraints can be met) can be delivered to regional lymph nodes that will not be resected at the time of surgery.
- Based on the data by Huang et al 3, dose to potentially resectable disease (that routinely removed in a modified radical mastectomy: breast and level I/II lymph nodes) will be limited to 54 Gy at 2 Gy/fraction or 57 Gy at 1.8/fraction to limit surgical complications in operable patients.

Dose regimen (of the three listed options above) will be at the discretion of the treating oncologist. Nearing the conclusion of 50-54/57/51 Gy patients will be reassessed for operability by the attending physicians and/or the surgeon.

Protocol based radiation dose to the CTV incorporates regimens in current practice. While an argument for a palliative dose strategy such as 30 Gy in 10 fractions of 3 Gy per fraction could be made for this population, there is little to no data using Capecitabine with fraction sizes larger than 2.0 Gy per fraction, and it has been suggested by the gastro-intestinal radiation service at MDACC 13 that higher dose is needed to effectively test a radiosensitizer. .

Treatment will be continued on the basis of tumor reassessment and/ or disease status

performed at 1 week intervals. All radiation planning will be peer-reviewed in a weekly breast radiation planning QA conference. Radiation to a second site (metastatic disease excluding whole brain) during or overlapping with protocol specified therapy is permitted.

4.2 Radiation Treatment Planning

Custom immobilization molds (cradle devices) and angle boards are recommended as appropriate per patient body habitus and site of disease. Patients will undergo CT simulation and this will be performed in the treatment position as identified by the attending Radiation Oncologist. Entry primary tumor size based on RECIST criteria will be evaluated on the planning CT obtained for simulation. Treating physician will be asked to enter this data onto a protocol specific form subsequent to the simulation.

When available, cross-sectional and ultrasound diagnostic imaging and reports obtained for clinical use and treatment planning independent of this study will be used to define regions of current and presenting gross disease in the breast and draining lymphatics. These will be contoured on the treatment planning CT. Gross disease at the time of simulation will be labelled gross target volume (GTV), complete radiographic response of prior gross nodal disease not radiographically detectable at the time of radiation planning will be contoured as clinical target volume (CTV). GTV and CTV will receive $\geq$90% of the protocol-specified dose (50-54 Gy at 2Gy per fraction, 57 Gy at 1.8 Gy per fraction or 51 Gy at 1.5 Gy per fraction bid). Treatment will be administered with high energy linear accelerators (6-18 MV) as determined by optimized treatment planning techniques. Treatment will be delivered using standard tangential irradiation techniques and these fields will be further optimized by use of cerrobend customized blocks and/or multi-leaf collimators. Dose limiting structures will be contoured and used to limit dose in treatment plans prescribed to $\geq$60 Gy (see below).

Standard 3D conformal treatment approaches previously described 14 and summarized below will be considered for all cases. IMRT may be used to achieve adequate coverage of targets. Standard 3D approach entails tangent fields with a coplanar posterior border to encompass the gross disease in the breast. The maximum distance from the posterior border of the tangent field to the chest-wall lung interface will be 2.5 cm anywhere within the field. When using a supraclavicular axillary apex field the dose will be calculated based upon anatomical considerations and location of gross disease by the attending Radiation Oncologist. When internal mammary nodes are treated the nodes will be treated with electrons, deep tangent technique or IMRT if normal tissue constraints can not be met without this technology. The dose using the electron technique should be calculated such that the 90% isodose line encompasses the nodes as determined by the depth to the internal mammary artery and vein.

4.3 Dosimetry

Dosimetry will be obtained by computerized dosimetry with lung inhomogeneity

correction. Wedge filters, tissue compensators, and field within field intensity modified radiotherapy may be used to minimize dose inhomogeneity. Dose volume histograms will not be mandated for 3D planning with dose $\leq$ 60 Gy.

Dose constraints for IMRT planning:

Total lung V20 < 35%,V5 < 65%;

mean lung dose < 20 Gy;

Heart V50 < 50%;

Spinal cord will be limited to 45 Gy maximum;

Brachial plexus will be limited to 66 Gy maximum, no more than 1 cc can receive 66 Gy, 100% cannot receive > 60 Gy.

4.4 Documentation

All CT datasets used for planning will be archived for documentation. Treatment plans will be entered into the electronic medical record as per standard practice protocol. Weekly quality assurance films will be taken of each radiation field and approved by the attending physician. These films will be saved in the electronic documentation and verify record: Mosaiq.

4.5 Treatment Interruptions

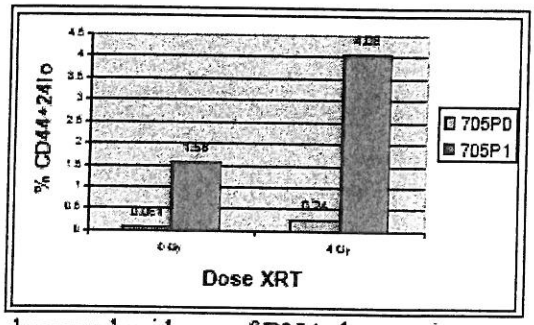
If a treatment interruption is necessary for acute radiation toxicity of the skin, aggressive treatment of the area with a product like Duoderm , Aquaphor, or Mepilex will be initiated to minimize the treatment break. Irradiation will be completed to the prescribed doses and the total number of fractions and the reasons for interruption of therapy will be recorded in the patient treatment record. Patient will be inevaluable if treatment break exceeds 1 week.

5.0 Biomarkers

5.1 Role of Cancer Stem Cells in Breast Cancer

According to the most stringent criteria, a marker deserving of the moniker "stem cell marker" should reliably identify a multipotent single cell capable of recapitulating the heterogeneity of the tumor from which it was derived. It should also be capable of self-renewal; that is, it should have the ability to divide asymmetrically and give rise to an exact replica of itself. It should have limitless replicative potential when needed, although the cell may be quiescent in the non-pathologic state. Unfortunately none of these criteria are easily measured or observed *in vivo*, thus creating the need for a surrogate marker or set of markers to identify these cells. Further complicating this effort is the reality that studying cells under *ex vivo* conditions may indeed change the profile of the markers 15. In addition, the expected rarity of these cells requires sensitive techniques to measure them. To date, there are few if any single markers that fulfill all of these criteria in human solid tumor stem cell biology. Many markers have been examined that fulfill some of these criteria and have therefore been dubbed "progenitor markers." These markers identify a subcategory of cells in the stem cell hierarchy that at this point have unclear clinical significance. To the stem cell purist, finding the progenitor is not the Holy Grail. Clinically, however, it is possible that some tumors may in fact result from self-renewing mutations in progenitors, which could make progenitor markers a meaningful biomarker for these tumors.

The existence of **human breast cancer** tumor-initiating cells was strongly bolstered by the landmark paper by Al-Hajj et al demonstrating the prospective identification of a population of human breast tumor initiating cells capable of recapitulating the phenotype of the human tumors from which they were derived when injected into the cleared mammary fatpad of a mouse [16]. Al-Hajj et al reported that as few as 200 $Lin^-CD44^-CD24^{-/lo}ESA^+$ cells derived from pleural effusions of patients with metastatic breast cancer were capable of regenerating tumors in contrast to thousands of cells lacking this phenotype that did not give rise to tumors. This work has, as expected, galvanized the field and, in spite of several limitations, has spawned the most literature to date leading towards a meaningful biomarker. Comparison of the results of a gene expression analysis of the $CD44^+CD24^{-/lo}$ cells from these cases with results for cells from normal mammary epithelium yielded a gene expression signature that predicted distant-metastasis free survival and overall survival in breast cancer as well as three other tumor types [17]. In preclinical studies, Phillips et al demonstrated that MCF7 cells with the $CD44^+CD24^{-/lo}$ phenotype were relatively resistant to radiation, generating fewer reactive oxygen species and decreased evidence of DNA damage in response to radiation [18]. In total, the $CD44^-CD24^{-/lo}$ phenotype exhibited several of the characteristics expected of a meaningful "cancer stem cell" biomarker when assayed by FACS analysis of freshly isolated cells. Cells expressing this phenotype from human metastatic pleural effusions are tumor-initiating, gene profiles from these cells predict for outcome in multiple tumor types, and in cell culture, cells expressing this phenotype are resistant to radiation. Preliminary studies in primary mammosphere culture from human pleural effusion cells suggest these findings of resistance to radiation in cell lines are clinically relevant and testable in fresh primary human material grown as mammospheres (Figure 2. left, primary mammospheres (705P0) and secondary/passaged mammospheres from the same patient (705P1) were irradiated ex vivo and $CD44^+CD24^{-/lo}$ assayed by flow cytometry).



5.2 Biomarkers (Circulating tumor cells)

Archived pathology specimens are clearly the most readily available human tumor samples, and antibody-based biomarkers amenable to immunohistochemical assays are desirable, but not easily converted from flow cytometry studies such as those described above. Efforts to correlate findings from an immunohistochemical analysis of CD44+24-/lo expression have demonstrated the challenge in using a multi-marker biomarker.

In the absence of a single marker that can be applied to immunohistochemistry, Balic et al employed spectral imaging in conjunction with double marker immunohistochemistry to examine the simultaneous expression of CD44 and CD24 on cytokeratin-positive, disseminated tumor cells in the bone marrow of patients with early stage breast cancer. They reported that this was a technically feasible approach in these samples, and detected CD44+CD24-/lo cells in all 50 samples with a median prevalence of 66% 19. The identification of this tumor-initiating phenotype on all cytokeratin positive disseminated tumor cells in the bone marrow raises interesting questions about the potential role for the detection of disseminated or circulating tumor cells as surrogate stem cell biomarkers. Circulating/disseminated tumor cells (C/DTCs) are tumor/epithelial cells in the blood or bone marrow of patients with breast cancer. In patients with metastatic breast cancer, the

presence of more than 5 of these cells in 7.5 mL of peripheral blood predicts for overall survival 20. These cells are detected by the presence of the epithelial cell marker CD326 (aka ESA or Ep-CAM), can be found in up to 30% of patients without known metastatic disease appreciated on standard staging studies even after systemic chemotherapy 21 and may predict for response to treatment 22. CTCs can be assayed in a standardized FDA approved assay and quantitated. In our own practice we have found that 75-90% of disseminated tumor cells in the bone marrow are CD44+24-/lo (unpublished data). More recent data has further refined the stem cell phenotype suggesting aldehyde dehydrogenase1 activity assayed as alde-fluor by flow cytometry yields greater selection of tumor initiating cells 23, and as such this single marker may be substituted for CD44+24lo studies.

5.3 CTCs as surrogate cancer stem cell markers

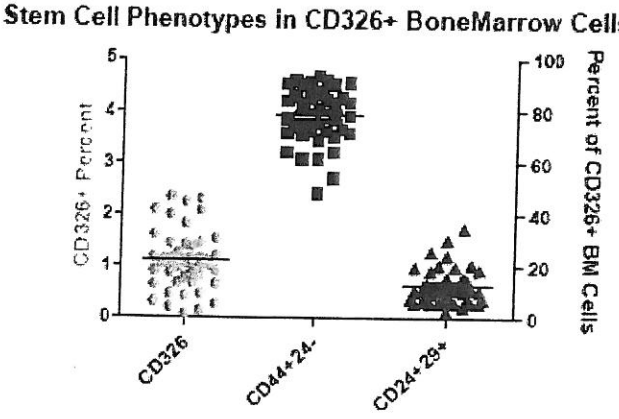
The field of solid tumor stem cell biology has re-emerged at the forefront of clinical oncology in recent years due largely to the identification of new, prospectively identified stem/progenitor cell markers. Numerous putative markers are currently under investigation and considerable work is being done to identify new ones. This process is beset by two main challenges: how robust are the criteria used to validate the identified cell as a stem cell, and to what degree, under what conditions, and in which patients are identification of these cells reproducible? Thus far, prevailing observations across disease sites are that cancer stem cells are often basal cells, devoid of markers of differentiation such as hormone receptors, and can exhibit similar adhesion molecule profiles, CD44+, and more recently ALDH1. Commonalities among tumor-initiating cell surface markers have facilitated tumor-initiating cell identification in multiple tumor sites; however, the impact of tissue digestion on marker specificity inhibits the use of established cell surface stem cell markers on intact tissue sections or archived tissue. Cell surface molecules while ideal targets for FACS analysis, may not provide the optimal targets for IHC which are critical to widespread biomarker use.

The circulating tumor cell (CTC) population is an appealing cancer stem cell surrogate as these cells need not be subjected to digestion for evaluation, and appear to express the markers representative of tumor initiating cells (Figure 3, unpublished data; ARC-MD), hypothetically because they are indeed seeking a niche in which to sustain new disease foci. Prior and ongoing studies have established the feasibility of collecting CTCs in the randomized trial setting, and technology is emerging to facilitate systematic examination and quantitation of these cells. Recently, the U.S. Food and Drug Administration (FDA) approved an assay for the detection of circulating tumor cells in peripheral blood using a semi-automated system, the CellSearch system, and other approaches not yet approved by the FDA are under investigation.

The translational component of this study will correlate the change in CTCs with chemo-radiation treatment to change in expression the best putative stem/progenitor markers on digested tumor tissue. This will assess number of CTCs as a surrogate

endpoint for treatment of cancer stem cells, appropriately collect and store tissue and serum for future correlation of tumor stem cell markers to this endpoint, and set the stage for incorporation of targeted stem cells therapies in the treatment of locally advanced IBC and non-IBC. This component of the trial will be optional. Samples will be processed and analyzed by the IBC stem cell core lab (Woodward WA, and Reuben, JM).

Figure 3. Bone marrow isolated on IRB approved protocol from breast cancer patients undergoing surgery for primary tumor. Tumor cells identified by CD326+CD45- were assessed for stem cell phenotypes by flow cytometry



%	% $CD326^{+}$ $CD45^{lo}$	% $CD44^{+}$ $CD24^{lo}$	%$CD24^{+}CD29^{+}$
Mean ± SEM	0.81 ± 0.07	76.3 ± 3.8	13.3 ± 2.4

6.0 Study Design

This is a single center, neoadjuvant phase II study (Schematic Appendix D). Eligible subjects must be women with a diagnosis of invasive breast cancer with primary or recurrent gross disease in the breast or chest wall or lymph nodes that is progressive, persistent, or minimally responsive to chemotherapy. Patients with oligometastatic disease (generally ≤ 3 distant sites, but at the discretion of the treating physician) who would benefit in terms of symptom palliation (pain, drainage, or emotional duress) are eligible. After the initial screening, patients who consent to translational studies will have the following samples stored or analyzed: serum (for CTCs, 1 tube – cell save; serum, 1 tube speckled top) and urine collection, and core needle biopsy (flow cytometry).

Size of tumor lesions in the breast and nodal basins will be documented based on initial clinical exam and imaging (planning CT) using recist criteria (Appendix F). All RECIST measurements will be made by a single physician, Dr. Woodward. IBC status will be recorded. Palliative vs pre-operative intent (based both on extent of metastatic disease and primary disease) will be recorded by the treating physician (Initial Assessment Form Appendix G). Subsequently, eligible, consenting women will receive pre-operative radiation with concurrent capecitabine. Capecitabine is commercially available and the commercially available supply will be used. Neither the drug nor the radiation will be free. Patients receiving Herceptin (trastuzumab) are eligible for participation and may receive concurrent Herceptin plus radiation and Xeloda (capecitabine). Chemotherapy administered after protocol treatment (after the last dose of radiation and capecitabine) is acceptable. Surgical consultation or follow up will be requested to be scheduled after 45 Gy and before the final fraction of radiation for patients treated with pre-operative intent. The treating oncologist may change this status to palliative if the clinical course makes the surgical consult unnecessary/patient is clearly unresectable in the judgment of the treating oncologist.

After completion of 45 Gy and within 15 days of completing treatment the total protocol specified dose to the breast, in patients who consent to translational studies a second core needle biopsy (for banking and flow cytometry), serum (for CTCs, serum) and urine collection will be performed.

Linear accelerator based CT imaging (CT on rails or cone beam CT) or re-CT-simulation will be performed after 45 Gy to assess for the need to re-plan secondary to response. Tumor size will be documented using recist criteria based on these images. Additional accelerator based CT imaging is acceptable as clinically indicated.

Definitive or palliative local therapy with surgery when indicated will be performed after completion of the combined regimens. If the patient is deemed operable the first follow-up visit will occur after surgery. If the patient is deemed inoperable the first follow-up visit will occur after concurrent Capecitabine and radiation therapy. Subjects will be followed every 3 months (+/- 1 month) post treatment times 1, then every 6 months (+/- 1 months) times 1, then every 12 months (+/- 2 month) times 2. PET/CT scan or ultrasound will be performed in conjunction with the first follow up visit. For patients treated to definitive dose for gross unresected disease (any treatment field receiving a total of > 60 Gy in the mosaic prescription), all sites receiving > 60 Gy will be scored as complete response, partial response, noresponse/progressive disease based on imaging and exam after this visit and when evaluable and all subsequent visits. Subsequent radiologic studies will be at the discretion of the treating oncologist as dictated by standard of care. Toxicity will be recorded at each visit using the RTOG criteria.

Capecitabine dose reduction criteria are as follows:

Capecitabine will be held for patients experiencing grade 3-4 Capecitabine related

toxicities until symptomatic improvement (Grade 1). Radiation therapy will not be held for Capecitabine related toxicities. Patients will then be restarted with reduced dose of Capecitabine (level-1). Level -1 (25% dose reduction) = 600 mg/m2 BID. If patients develop additional grade 3-4 toxicity in spite of dose reduction, the chemotherapy will be permanently discontinued.

Correlation will be made between Change in CTC level and change in percent of CD44+CD24lo or ALDH1+ cells in the tumor biopsy. Change will be correlated with clinical and pathologic response (in patients who undergo surgery)

Approximately 60 subjects will be enrolled. Assuming an average accrual rate of 3-4 subjects per month, the duration of accrual will be approximately 20 to 26 months.

Biomarkers Studies

- **Material Collected (will be omitted if the same sample is to be collected and stored at the same visit for another protocol, ie IBC registry or 2007-0818):**
- *Pre-treatment: Sonogram-guided core biopsy of tumor mass, CTCs collected in cell search tube, serum, urine.*
- *After completion of 50 Gy (qd) or 45 Gy (bid) and within 15 days of completing treatment the total protocol specified dose to the breast, : sonogram-guided core biopsy, CTCs, serum, urine*

- **Proposed Studies:**
- 1. Serum and urine will be collected and stored for future translational studies.
- 2. Multiple cores not greater than 5 will be obtained at the time of each biopsy. Total biopsy number will be at the discretion of the performing physician and studies below will be prioritized in the order they are listed when sufficient material for all is not collected. Paired biopsy specimens (pre and post initiation of therapy) will be treated as follows:

- Pair 1 and 2: Core material will be delivered fresh to IBC core laboratory. Material will be digested to single cells using established protocols for flow cytometry and/or mammosphere culture 24. Mammosphere efficiency will be correlated to percent stem cell marker expression as well as to residual tumor burden determined pathologically at the time of surgery. Conditioned media from mammosphere cultures may be examined using proteomic analysis for secreted proteins that are selectively regulated after therapy and that are common across patient samples. If material is sufficient, mesenchymal stem cells will be cultured and correlated to CTCs.
- Pair 3: Microarray analysis will be performed on the entire specimen. Significantly upregulated or down-regulated genes will be correlated to known clinical variables as well as the primary endpoints.

- Pair 4: Material will be transplanted into the cleared mammary fatpads of nod/scid mice to maintain tissue samples and generate new material for additional studies. Tissue fragments form all tumors generated will be frozen.
- Pair 5: Formalin embedded for future biomarker studies. Immunohistochemical analysis for predictive markers related to Capecitabine mechanism (TS, TP, DPT, HNCT1) will be performed post-hoc on these samples and or archived tissue from the primary diagnosis when this core is not collected.

7.0 Study Design Discussion

This single arm single institution, open-label, phase II trial will provide sufficient data to define and quantitate the safety and efficacy of the combination of neo-adjuvant concurrent radiation and capecitabine in select locally advanced breast cancer patients.

8.0 Selection of Patients

1. **8.1 Inclusion Criteria**

1. Histological confirmation of invasive breast cancer
2. No contraindications to receiving a course of radiation treatment (pregnancy, prior radiation to the volume with disease, or systemic disease in which radiation therapy is an absolute contraindication).
3. Patients who have chemo-refractory gross disease in the breast causing symptoms (pain, drainage, duress) OR

   gross disease in the breast ($\geq$ T3) and/or lymph node(s) progressive, persistent, or minimally responsive to chemotherapy deemed inoperable or questionable inoperable OR

   recurrent gross disease in a previously unirradiated breast or on the chest wall or in the regional lymphatics (core biopsy will not be offered to patients without gross disease in the breast).

4. Are able to swallow and retain oral medication (intact pill).
5. Age over 18.
6. Female gender

8.2 Exclusion Criteria

1. Have an active or uncontrolled infection.
2. Have dementia, altered mental status, or any psychiatric condition that would prohibit the understanding or rendering of informed consent.
3. Have used an investigational drug within 21 days preceding the first dose of study medication.
4. Are receiving therapeutic anti-coagulation therapy (i.e. warfarin, heparin).
5. Uncontrolled arrhythmia or history of CHF based on clinical history or physical exam.
6. Patient cannot receive whole brain irradiation concurrently with Xeloda treatment.

9.0 Withdrawal Criteria

9.0 Withdrawal Criteria

A subject may voluntarily discontinue participation in this study at any time. The investigator may also, at his or her discretion, discontinue the subject from participating in this study at any time. In addition, study medication will be discontinued if unmanageable toxicity is documented, or the subject becomes pregnant.

If the subject is discontinued from participation in the study for any reason, the investigator must make every effort to perform the evaluations as shown under 'Withdrawal' in the respective Time and Events Table (Appendix E).

A subject will also be taken off study treatment if any of the following occur:

9.1 During Radiation Treatment:

1. Locoregional disease progression.
2. Treatment is interrupted for more than 1 consecutive week.
3. An intercurrent illness, which would in the judgment of the Study Investigator, affect assessments of clinical status to a significant degree or require discontinuation of study treatment.
4. Nonprotocol therapy (chemotherapy) is administered during study treatment.
5. Non-compliance with protocol or treatment.
6. Refuses to continue treatment. Subject will undergo surgery; toxicity data will continue to be collected.

9.2 Subjects will be considered off study if any of the following occur:

1. Withdrawal of consent (subject will not be contacted and no further information will be collected).
2. Death.

Subjects who are taken off study treatment or who are considered to be off study will not be replaced.

10.0 Endpoints

Primary

- Response evaluated by RECIST criteria (APPENDIX F)

i. Secondary

The secondary efficacy objectives of the study are:

- To determine the rate of conversion to operable and cCR and pCR after completion

of all protocol specified therapy among patients who go on to surgery. This includes the following:

- Pre-operative concurrent radiation with capecitabine to the breast and at risk or involved regional lymph node basins
- To determine locoregional control of unresected nodal disease treated to definitive radiation dose (≥ 60 Gy)

ii. Safety

The secondary safety objectives of the study are:

- Determine the rate of post-surgical wound complications after pre-operative radiation and capecitabine
- Determine the rate of grade III toxicities (excluding acute skin toxicity)

iii. Translational endpoint:

The biomarker research objectives of the study are:

- To determine if change in the absolute number of circulating tumor cells (CTCs) in the blood during pre-operative radiation with or without chemotherapy correlates to the percentage of viable cells in a tumor core biopsy expressing putative stem/progenitor cell markers pre and post therapy. Patients whose gross disease cannot be completely encompassed by a combination of locoregional therapy with chemo/HOU/UTMDACCiation and surgery will be analyzed separately.

The Time and Events Table is included in Appendix E.

11.0 Power Calculations and Statistical Analysis

11.0 Power Calculations & Statistical Analysis

11.1 Hypotheses

The primary objective of this study is to determine response rate after completion of all protocol specified neoadjuvant chemotherapy+ concurrent radiation. The regimen will be considered of interest for further study if the response rate is at least 80% and the grade III toxicity excluding acute skin toxicity is <20%.

11.2 Treatment Comparisons

As all eligible patients will receive the same treatment, there will be no comparisons between treatments.

11.3 Sample Size Considerations and Interim Analyses

The primary objective of this study is to determine the efficacy of pre-operative radiation with concurrent capecitabine in patients who have experienced a minimal response or progression after standard neoadjuvant chemotherapy. Efficacy will be determined by tumour response (complete response + partial response) as measured by RECIST criteria. Tumour response will be assessed after 45 Gy. Those who do not finish will be considered non-responders. Toxicity will be assessed at the same time. Surgical complications will be assessed independently within 6 weeks of surgery. Only grade three toxicity will be considered in the stopping rules. This study will be monitored for both efficacy and safety. Safety will be determined by the rate of grade 3 or higher toxicities, excluding acute skin toxicity. This regimen will not be considered of interest for further study if there is a high probability that the response rate is less than 80% or the severe toxicity rate is greater than 20%. Priors regarding response are based on combined retrospective data from three studies of 135 total patients treated for gross disease in the breast at our institution and detailed in the background (sections 3.2 and 3.5). Local control (a surrogate for response) at 5-years in patients treated for gross IBC disease in the breast with radiation alone was 75% (N = 42, unpublished). In 38 similar patients with non-inflammatory breast cancer local control was 70% at 5 years2. In an unpublished retrospective analysis of 55 patients treated with the regimen proposed herein, 5-year local control was 85% (Perkins, SABCS abstract, 2007). There are limited and poor data regarding radiation alone toxicity in this exact cohort. The priors for toxicity were based on aggressive xrt in post-mastectomy patients (N = 192)14. In these patients, we expect a 15% rate of Grade 3 toxicities in patients receiving 60Gy and a 29% rate of Grade 3 toxicities in patients receiving 66Gy 2,14, and unpublished data. Clinically we are currently limiting the use of the latter regimen based on the impression that this is unacceptably high toxicity, so we are targeting a Grade 3 toxicity rate of no more than 20%. All patients who receive any treatment will be included in the analyses of both efficacy and safety. Patients who are not evaluable for response will be considered non-responders. The final analysis will be an Intent to Treat analysis. A total of 60 patients will be enrolled at the rate of 3-4 patients per month. Stopping boundaries will be assessed in cohorts of 5 patients after a minimum of 10 patients have been enrolled. Response and toxicity will be monitored independently. The method of Thall and Simon will be employed to perform interim monitoring.

11.3.1 Efficacy and Safety Monitoring

R,E is the probability of response with the study regimen and its assumed prior distribution is beta(1.6, 0.4). R,S is the targeted response rate and its assumed prior distribution is beta (80, 20). The trial will be stopped early if $Pr[R,E < R,S \mid data] > 0.95$. T,E is the probability of severe toxicity from the study regimen and T,S is the targeted rate of severe toxicity. The assumed prior distribution of T,E is beta (0.4, 1.6) and the assumed prior distribution of T,S is beta (20, 80). The trial will be stopped early if $Pr[T,E > T,S \mid data] > 0.925$.

The stopping boundaries corresponding to above probability criteria are:

Number of Patients Evaluated	Number of Responses <=	Number of Toxicities >=
10	5	5
15	8	6
20	12	8
25	15	9
30	19	11
35	22	12
40	26	13
45	30	15
50	33	16
55	37	17
60	40	19

11.3.3 Operating Characteristics

The following table summarizes the probability of stopping early, the expected study size, number of responses, and number of toxicities for the indicated true response and toxicity probabilities:

True Respor Probability	True Toxicit Probability	Probability t trial stops ea	Average Nu of Patients	Average Nu of Responses	Average Nu of Toxicitie
0.65	0.10	0.78	31.8	20.7	3.2
0.65	0.20	0.81	30.0	19.5	6.0
0.65	0.30	0.92	23.4	15.2	7.0
0.80	0.10	0.08	56.7	45.4	5.7
0.80	0.20	0.19	52.6	42.0	10.5
0.80	0.30	0.66	36.4	29.1	10.9
0.95	0.10	0.003	59.8	56.8	6.0
0.95	0.20	0.12	55.4	52.6	11.1
0.95	0.30	0.63	38.0	36.1	11.4

At the end of the study, we will estimate the posterior distribution of the response rate and severe toxicity rate. If the trial is not stopped early and 54 responses are observed, the 95% posterior credible interval for response will be 81.1%, 95.9%. If the trial is not stopped early and 5 patients experience severe toxicity, the 95% posterior credible interval for severe toxicity will be 3.1%, 16.8%.

The primary translational endpoint of this study is the change in the stem cell population as measured by flow cytometry from before to after radiation treatment. The change in each measured biomarker will be summarized with standard descriptive statistics such as mean, median, standard deviation, and range. We will determine the association between biomarkers and the change in biomarkers with tumour response and CTC status (present/absent) with a t-test or Wilcoxon's rank sum test as appropriate. All analyses will be repeated in the subgroup of patients diagnosed with inflammatory breast cancer (IBC).

11.4 Sample Size Re-estimation

Sample size re-estimation is not planned for this study.

11.5 Analysis Populations

Analyses of both efficacy and safety will be performed in the intent-to-treat (ITT) population, and will comprise all patients who received at least one dose of the investigational product. The surgical population includes only subjects who underwent definitive surgery.

11.6 Efficacy Analysis

The ITT population will be used for the primary analysis of both the efficacy and safety data. The surgical population will be used for a secondary analysis of the surgical efficacy data. Only patients who undergo both pre-treatment and post-treatment core biopsies will be considered in the primary translational analysis.

11.6.1 Primary Analysis

The primary endpoint in this study is response defined as the percentage of subjects achieving a complete or partial tumor response using RECIST criteria. The response rate will be calculated from the view of best response that records confirmed cases of CR or PR. Subjects with unknown or missing response will be treated as non-responders; i.e., they will be included in the denominator when calculating the percentage. Response will be evaluated by the recist criteria 4 weeks (+/- 4 days) after completion of radiation therapy.

11.6.2 Secondary Analysis

Patients who were deemed at time of enrolment to be inoperable or questionably inoperable (requiring flap closure with doubt regarding ability to obtain negative margins based on surgical oncology assessment) who have objective clinical response and receive mastectomy or are offered mastectomy but refuse will be considered having converted to operable. Patients who are resectable but considered inoperable based on advanced locoregional disease or M1 disease who have a mastectomy and no FDG-avid disease on PET at 3 month follow up will also be considered having converted to operable.

A secondary indicator of treatment efficacy is pCR. pCR is defined as the lack of any invasive disease in the breast and lymph nodes. A pathologist at the University of Texas M.D. Anderson Cancer Center will perform accurate sampling and review of all cases that have surgery. pCR will be assessed at the time of surgery following the completion of all protocol specified neoadjuvant chemotherapy, which will be approximately 26

weeks following the start of neoadjuvant chemotherapy. Subjects with unknown or missing response and those who do not convert to operable will be included in the primary efficacy analysis as non-responders.

A secondary analysis will calculate response rate based on subjects with evaluable tumor response only; i.e., the denominator will only include subjects with CR, PR, SD, or PD and will not include those with unknown or missing best response.

Exact 95% confidence limits for the clinical response rate will be calculated.

11.7 Safety Analyses

The ITT population will be used for the analysis of safety data.

12.0 References

1. Gaui MF, Amorim G, Arcuri RA, *et al.* A phase II study of second-line neoadjuvant chemotherapy with capecitabine and radiation therapy for anthracycline-resistant locally advanced breast cancer. *Am J Clin Oncol* 2007;30:78-81.
2. Liauw SL, Benda RK, Morris CG, *et al.* Inflammatory breast carcinoma: outcomes with trimodality therapy for nonmetastatic disease. *Cancer* 2004;100:920-928.
3. Huang E, McNeese MD, Strom EA, *et al.* Locoregional treatment outcomes for inoperable anthracycline-resistant breast cancer. *Int J Radiat Oncol Biol Phys* 2002;53:1225-1233.
4. Ishikawa T, Utoh M, Sawada N, *et al.* Tumor selective delivery of 5-fluorouracil by capecitabine, a new oral fluoropyrimidine carbamate, in human cancer xenografts. *Biochem Pharmacol* 1998;55:1091-1097.
5. Sawada N, Ishikawa T, Sekiguchi F, *et al.* X-ray irradiation induces thymidine phosphorylase and enhances the efficacy of capecitabine (Xeloda) in human cancer xenografts. *Clin Cancer Res* 1999;5:2948-2953.
6. Liauw SL, Minsky BD. The use of capecitabine in the combined-modality therapy for rectal cancer. *Clin Colorectal Cancer* 2008;7:99-104.
7. Ben-Josef E. Capecitabine and radiotherapy as neoadjuvant treatment for rectal cancer. *Am J Clin Oncol* 2007;30:649-655.
8. Dunst J, Reese T, Sutter T, *et al.* Phase I trial evaluating the concurrent combination of radiotherapy and capecitabine in rectal cancer. *J Clin Oncol* 2002;20:3983-3991.
9. Ngan SY, Michael M, Mackay J, *et al.* A phase I trial of preoperative radiotherapy and capecitabine for locally advanced, potentially resectable rectal cancer. *Br J Cancer* 2004;91:1019-1024.

10. Dunst J, Debus J, Rudat V, *et al.* Neoadjuvant Capecitabine Combined with Standard Radiotherapy in Patients with Locally Advanced Rectal Cancer : Mature Results of a Phase II Trial*. *Strahlenther Onkol* 2008;184:450-456.
11. Krishnan S, Janjan NA, Skibber JM, *et al.* Phase II study of capecitabine (Xeloda) and concomitant boost radiotherapy in patients with locally advanced rectal cancer. *Int J Radiat Oncol Biol Phys* 2006;66:762-771.
12. Mosteller RD. Simplified calculation of body-surface area. *N Engl J Med* 1987;317:1098.
13. Crane CH, Ellis LM, Abbruzzese JL, *et al.* Phase I trial evaluating the safety of bevacizumab with concurrent radiotherapy and capecitabine in locally advanced pancreatic cancer. *J Clin Oncol* 2006;24:1145-1151.
14. Woodward WA, Buchholz TA. The role of locoregional therapy in inflammatory breast cancer. *Semin Oncol* 2008;35:78-86.
15. Liao MJ, Zhang CC, Zhou B, *et al.* Enrichment of a population of mammary gland cells that form mammospheres and have in vivo repopulating activity. *Cancer Res* 2007;67:8131-8138.
16. Al-Hajj M, Wicha MS, Benito-Hernandez A, *et al.* Prospective identification of tumorigenic breast cancer cells. *Proc Natl Acad Sci U S A* 2003;100:3983-3988.
17. Liu R, Wang X, Chen GY, *et al.* The prognostic role of a gene signature from tumorigenic breast-cancer cells. *N Engl J Med* 2007;356:217-226.
18. Phillips TM, McBride WH, Pajonk F. The response of CD24(-/low)/CD44+ breast cancer-initiating cells to radiation. *J Natl Cancer Inst* 2006;98:1777-1785.
19. Balic M, Lin H, Young L, *et al.* Most early disseminated cancer cells detected in bone marrow of breast cancer patients have a putative breast cancer stem cell phenotype. *Clin Cancer Res* 2006;12:5615-5621.
20. Cristofanilli M, Budd GT, Ellis MJ, *et al.* Circulating tumor cells, disease progression, and survival in metastatic breast cancer. *N Engl J Med* 2004;351:781-791.
21. Pantel K, Brakenhoff RH, Brandt B. Detection, clinical relevance and specific biological properties of disseminating tumour cells. *Nat Rev Cancer* 2008;8:329-340.
22. Camara O, Rengsberger M, Egbe A, *et al.* The relevance of circulating epithelial tumor cells (CETC) for therapy monitoring during neoadjuvant (primary systemic) chemotherapy in breast cancer. *Ann Oncol* 2007;18:1484-1492.
23. Ginestier C, Hur MH, Charafe-Jauffret E, *et al.* ALDH1 is a marker of normal and malignant human mammary stem cells and a predictor of poor clinical outcome. *Cell Stem Cell* 2007;1:555-567.
24. Dontu G, Abdallah WM, Foley JM, *et al.* In vitro propagation and transcriptional profiling of human mammary stem/progenitor cells. *Genes Dev* 2003;17:1253-1270.
25. Thall, PF and Simon, R. "A Bayesian approach to establishing sample size and monitoring criteria for phase II clinical trials", Controlled Clinical Trials 1994; 15:463-481.