A Randomized Phase II Neoadjuvant Study of Sequential Eribulin Followed by FAC/FEC-regimen Compared to Sequential Paclitaxel Followed by FAC/FEC-regimen in Patients with Early Stage Breast Cancer Not Overexpressing HER-2

Study Chair: Vicente Valero, MD, F.A.C.P.
Professor, Department of Breast Medical Oncology
The University of Texas MD Anderson Cancer Center
1515 Holcombe Boulevard, Unit 1354
Houston, TX 77030
(713) 792-2817 tel.
(713) 794 4385 fax.
vvalero@mdanderson.org

Collaborators:

Savitri Krishnamurthy, MD
Professor, Department of Pathology
The University of Texas MD Anderson Cancer Center

James M Reuben, MBA, PhD
Professor, Department of Hematopathology - Research
The University of Texas MD Anderson Cancer Center

Joe Ensor, PhD
Research Statistician, Department of Biostatistics
The University of Texas MD Anderson Cancer Center

Gary J Whitman, MD
Professor, Department of Diagnostic Radiology
The University of Texas MD Anderson Cancer Center

Naoto T Ueno, PhD
Professor, Department of Breast Medical Oncology
The University of Texas MD Anderson Cancer Center

Stacy Moulder, MD
Associate Professor, Department of Breast Medical Oncology
The University of Texas MD Anderson Cancer Center

Table of Contents

List of Abbreviations

AE	Adverse Event
ALT	alanine aminotransferase
AST	aspartate aminotransferase
b.i.d.	bis in diem/twice a day
CRF	Case Report/Record Form
CS&E	Clinical Safety and Epidemiology
CR	Clinical Research
CRO	Contract Research Organization
ECG	Electrocardiogram
IEC	Independent Ethics Committee
i.v.	intravenous(ly)
IRB	Institutional Review Board
o.d.	omnia die/once a day
p.o.	per os/by mouth/orally
REB	Research Ethics Board
SAE	Serious Adverse Event
SOP	Standard Operating Procedure
WHO	World Health Organization

1. OBJECTIVES AND RESEARCH HYPOTHESIS

1.1 Primary Objectives

To estimate the pathological complete response (pCR) rate of eribulin followed by FAC/FEC-regimen relative to the pCR rate of paclitaxel followed by FAC/FEC-regimen in patients with HER-2 negative, operable breast cancer.

1.2 Secondary Objectives

1.2.1 To evaluate the safety of the combination of eribulin followed by FAC/FEC-regimen.

1.2.2 To determine the rate of breast conservation surgery.

1.3 Exploratory Objectives

1.3.1 To explore in this population using Hot Spot Mutation Analysis (HSMA) and Molecular Inversion Probes (MIP) arrays and analyze if there are any predictive patterns of expression that may be differentially predictive of pCR to eribulin followed by FAC/FEC regimen versus paclitaxel followed by FAC/FEC regimen.

1.3.2 To determine the effect of eribulin on the presence of Circulating tumor cells (CTC) and CTCs with epithelial and/or EMT gene expression in PB.

1.4 Research Hypothesis

Sequential administration of eribulin followed by FAC/FEC-regimen (5-Fluorouracil, Adriamycin or Epirubicin, and Cyclophosphamide), has greater activity based on pCR rate relative to sequential administration of Paclitaxel followed by FAC/FEC-regimen as primary systemic therapy for women with early stage breast cancer.

A biomarker-defined population can be identified in which a higher pCR rate is observed in subjects treated with eribulin followed by FAC/FEC-regimen compared with paclitaxel followed by FAC/FEC-regimen.

2. BACKGROUND

2.1. Breast Cancer

Invasive breast cancer is the most common malignancy in women worldwide. In the United States, breast cancer is the most common female cancer, the second most common cause of death in women (after lung cancer), and is the main cause of death in women between the ages of 45 and 55. [2] However, over the last decade the mortality rate has declined in the United States and United Kingdom largely because of widespread use of mammography, breast cancer screening programs, advances in evaluation technique, and more effective adjuvant treatments.[3]

Studies that compared preoperative (neoadjuvant or primary systemic therapy) and adjuvant chemotherapy in patients with early stage breast cancer have shown no difference in overall survival or disease free survival. [4] Primary systemic therapy (PST) is increasingly being used in the management of patients with early breast cancer. Neoadjuvant chemotherapy allows for monitoring of response to chemotherapy and enhances chances of breast conservation surgery and/or a better cosmetic outcome following mastectomy in patients with locally advanced breast cancer. [5, 6] Additionally, the neoadjuvant setting is ideal for pharmacogenomic studies to identify breast cancer patients likely to respond best to therapy. The provision of a surgical specimen, at the end of therapy, allows for a more rapid assessment of response (pathological responses) than adjuvant trials. Pathological complete response (pCR) is widely accepted as a valuable prognostic indicator of long-term outcome after neoadjuvant therapy. [7]

2.2. Primary Systemic Therapy: Historical Perspective

The rationale for considering the evaluation of primary systemic therapy (PST) in patients with operable breast cancer began to evolve as clinical observations demonstrated the utility of this approach in patients with locally advanced breast cancer (LABC), [8-10] and inflammatory breast cancer (IBC). In addition, preclinical observations, [11, 12] and mathematical models of tumor growth, dissemination, and development of resistance to chemotherapy support the use of PST rather than adjuvant therapy. These could lead to achieve longer disease-free survival (DFS) and overall survival (OS), presumably through early treatment of systemic micromestatatic disease. Since its initial use in the early 1970s, PST has become the standard of care for management of LABC and IBC, and increasingly been used for treatment of large operable and more recently for early-stage breast cancer.

Chemo-, hormone-, and recently trastuzumab-base therapy are potential PST options for the different sub-types of breast cancer in 2011. PST provides several advantages, including down-staging allowing surgery for non-operable breast cancer, and increasing breast-conservative surgery rate in patients with large operable breast cancer. It also provides an early surrogate factor, pCR, for long-term outcome and in-vivo model to assess clinical benefit and finally a research tool for understanding breast cancer biology and treatment mechanisms of action(s).

2.3. Pathological Assessment

A variety of endpoints can be used to measure outcomes of PST for breast cancer other than directly measuring survival (DFS, and OS), which requires a large number of patients and long term follow-up. These endpoints included clinical response, radiologic response, rate of breast conservative surgery (BCS), and pathologic response. The results of several studies have been shown that pCR is predictive of long-term survival. [13-16]At present, the achievement of pCR has emerged as the primary end point of most interest in the clinical research literature. Attainment of pCR is associated with a favorable prognosis; such patients have a far lower risk of subsequent recurrence than do patients with residual invasive tumor at the time of surgery, and also seem to have improved overall survival. Despite the strong evidence of predictive value of pCR in this context, there is no consensus on the measurement of this important endpoint. Clinical and pathological responses are both frequently used as objective measurements of effectiveness of PST. Three of the most commonly used criteria in the literature are those by Sataloff et al [16], Feldman et al, [13] and most recently Symmans and collaborators from the University of Texas, MD Anderson Cancer Center. The first 2 sets of criteria have some overlap but, for the most part, differ from each other.

In general, 60% to 90% of patients with invasive breast cancer show clinical response; however, only 3% to 30% of patients achieve pCR. Two large studies using PST, NSABP B-18 and B-27 defined pCR as no residual invasive cancer in the breast after PST and at the time of surgery, whereas other studies also take node status and noninvasive cancer into account. An International Expert Panel recently recommended that pCR be defined as no invasive or noninvasive tumors in the beast and axillary tissues removed at the time of surgery. [8]

Symmans et al [17] showed a continuous index combining pathologic measurements of the primary tumor (size and cellularity) and nodal metastases (number and size) and tested as an independent predictor of distant relapse-free survival. Patients with minimal residual disease (RD) (RCB-I) carried the same prognosis as pCR (RCB-0). On the other hand, patients with extensive RD (RCB-III) had poor prognosis. RCB was independently prognostic in a multivariate model that included age, pretreatment clinical stage, hormone receptor status, hormone therapy, and pathologic response (pathologic complete response [pCR] vs. RD; hazard ratio = 2.50; 95% CI 1.70 to 3.69; $P < .001$). Seventeen per cent of patients had minimal RD (RCB-I). These patients carried the same prognosis as pCR (RCB-0). Extensive RD (RCB-III) was seen in 13% of patients. It was associated with poor prognosis, regardless of hormone receptor status, adjuvant hormone therapy, or pathologic American Joint Committee on Cancer stage of residual disease. The calculation formula and detailed description can be found at a dedicated Web site: http://www.mdanderson.org/breastcenter_RCB.

2.4. Phase II and III Randomized PST Breast Cancer Trials

Several large Phase III trials investigated the efficacy of chemotherapy when is administered as PST compared with adjuvant systemic treatment.

In 1998 the National Surgical Adjuvant Breast and Bowel Project (NSABP) conducted a large phase III study (NSABP P-18) to compare PST and post-operative chemotherapy. [7, 18, 19]A total of 1,523 patients with T1-3 N0-1 M0 breast cancer were randomized to receive four cycles of doxorubicin and cyclophosphamide (AC) either as PST or adjuvant therapy. Breast tumor size was reduced in 79% of patient after PST, and 36% had a clinical complete response (cCR) rate, 43% clinical partial response (cPR), and a 13% pCR. Clinical nodal response was observed in

89% of patients with node-positive disease; 73% had nodal cCR, and 44% of these patients had pCR. At 9 years, the authors reported no difference in DFS (67% for both groups) or OS (69% PST vs. 70% adjuvant groups; $P = .80$). However, there was a favor trend in favor of PST in women less than 50 years old (HR 0.85, $P = .053$). The investigators reported that the use of PST improved BCS from 60% to 67% ($P < .01$). Even with improved rates of BCS, there was no statistically significant difference in the rate of local recurrence between treatment groups ($P = .12$). A marginal increase in the rate of local recurrence for patients who were converted from proposed mastectomy to segmental mastectomy (15.9%) was seen when compared with patients who were eligible to undergo segmental mastectomy as per initial plan (9.9%) ($P = .04$). This difference loses statistical significance after controlling for age and initial clinical tumor size.
NSABP B-27, a large prospective randomized trial, [15, 20] was designed to evaluate whether the addition of docetaxel to AC PST would prolong DFS and OS and improve clinical and pathologic tumor response rates. Women with operable breast cancer (n = 2,411) were randomly assigned to receive either 4 cycles of PST AC followed by surgery (Group 1), 4 cycles of AC followed by 4 cycles of docetaxel, followed by surgery (Group 2), or 4 cycles of AC followed by surgery and then 4 cycles of docetaxel (Group 3). The addition of docetaxel to AC increased pCR rate (26.1% vs 13.7%; $P < .001$). pCR was a significant predictor of OS (HR 0.33, $P < .0001$). The pathologic nodal status after chemotherapy was also a significant prognostic factor for OS ($P < .0001$). However, this study did not prospective assess the role docetaxel in patients with residual disease after PST AC. There was no stratification after AC. The patients with all eight cycles of PST administered at front had a trend toward improvement in RFS. [15] One caveat with this study is that at the time it was conducted, all patients received tamoxifen, which was initiated concurrently with chemotherapy, regardless of hormone-receptor status. The simultaneous administration of tamoxifen and chemotherapy may have affect benefit from chemotherapy.
The European Organization for Research and Treatment of Cancer (EORTC) Trial 10902 randomized 698 patients with stage I to IIIB breast cancer to receive four cycles of PST or adjuvant FEC-100. [21] The primary objective of this study was to determine the impact of timing of therapy on DFS and OS. After a follow-up of 4-years the OS was 82% for PST group compared to 84% for those treated in the adjuvant setting ($P = .38$). For patient who received PST, the overall response rate (ORR) (cCR + cPR) was 49% and cCR 7%. Thirteen of 350 patients (4%) in the PST group had a pCR. For this study, response was determined by both clinical examination and changes with the mammogram, possibly explaining the low overall clinical CR. PST was associated with an increased rate of 35% BCS compared to 22% for the control group. The rate of locoregional recurrence was equivalent between treatment groups.
The European Cooperative Trial in Operable (ECTO) Breast Cancer randomly tested the efficacy of postoperative chemotherapy doxorubicin followed by cyclophosphamide, methotrexate, and 5-fluorouracil (CMF) or doxorubicin and paclitaxel (AP) followed by CMF versus PST consisting in AP followed by CMF. [22, 23] A total of 1,355 patients entered the study. Overall, PST induced a clinical response in 78% of the patients and pCR 23%. There was no significance in RFS when AP/CMF was given before surgery compared with the same regimen given after surgery (HR, 1.21; $P = .18$) However, the rate of breast-conserving surgery was significantly higher with preoperative chemotherapy (63% vs. 34%; $P < .001$)

The wide variety of PST clinical trials recently completed or ongoing in early breast cancer reflects the pressing need to identify the most effective agents and regiments to optimize both surgical and long-term outcomes for these patients.

Two prospective clinical studies from M.D. Anderson Cancer Center evaluating different sequence of taxane followed by anthracycline-based PST have been published. Green et al [24] evaluated the role of paclitaxel given at two different schedules in the PST setting. In this study, 258 patients were randomly assigned to receive weekly paclitaxel or standard every 3-weekly paclitaxel to determine if different schedules or dose densities of paclitaxel would achieve improved pCR rate. The doses of weekly paclitaxel varied based on clinical status of axillary nodes. Weekly paclitaxel was given at a dose of 80 mg/m2 for 12 weeks to those with node-negative disease or 150 mg/m2 (3 weeks on and 1 week off) for four cycles to those with node-positive disease. Standard paclitaxel was administered as 24-hour infusion at 225 mg/m2 every 3 weeks for four cycles. After completion of paclitaxel, all patients received FAC x 4 cycles. Clinical responses were similar in both groups (P = .25). The pCR rates were higher in those who received weekly paclitaxel than those who received standard paclitaxel (28% vs.15.7%; P = .02) with an improved BC rate (47% vs. 38%; P = .05). There are already data supporting the superiority of weekly paclitaxel over every 3-weekly paclitaxel in the adjuvant setting reported in the Eastern Cooperative Oncology Group (ECOG) 1199 [25] and in the metastatic setting reported in the Cancer and Leukemia Group B (CALGB) 9840. [26]

Lastly, Buzdar el al [27] compared two taxane schedules (weekly paclitaxel [WP] x 12 versus 3-week docetaxel plus capecitabine (DC) x 4 cycles, followed by: FEC-100 x 4 cycles. Patients were randomized 1:1 and stratified by the timing of therapy (PST vs. adjuvant). A total of 216 patients were treated with PST and 107 were randomized to WP arm and 109 to DC arm. The pCR rates were 18.7% and 17.4% on each arm, respectively (P = .81). The DC arm had higher incidence of hand foot syndrome, and myelosuppression, and WP treatment higher neurotoxicity. The primary endpoint was DFS, and the secondary endpoint pCR. The study was designated to include 930 patients to have 80% power. An interim analysis in June 2008 to the data monitoring committee the study was closed due to futility. The authors concluded that WP and DC in the PST setting had same efficacy and WP was associated with better tolerance and less toxicity.

2.5. Current Primary Systemic Chemotherapy Regimens

Pathological complete response rates are generally higher with anthracycline-based combinations than with regimens not containing anthracycline (doxorubicin or Epirubicin). Consequently, most PST regimens for breast cancer are anthracycline-based combination: AC (doxorubicin/Cyclophosphamide); FAC (fluorouracil/doxorubicin/cyclophosphamide); CE (cyclophosphamide/epirubicin); and FEC (fluorouracil/epirubicin/cyclophosphamide). However, other non-anthracycline based drug combinations, such as CMF (cyclophosphamide/methotrexate/fluorouracil) with or without a taxane, are also in common use. Increased duration of chemotherapy administration from 12 to 18 weeks or longer improves pCR rates. [6] The addition of paclitaxel or docetaxel to anthracyclines based regimes has resulted in pCR of up to 28.2%. [24] The Aberdeen study showed that tumors that did not respond to an anthracycline-based regimen may respond to docetaxel. Additionally sequential and non-concomitant addition of taxane to anthracycline-based chemotherapy results in higher pCR rates. [28]

At present, there is no evidence to suggest that one taxane is superior to the other in the neoadjuvant setting.
Table 1 shows multiple studies in the literature reporting pCR rates

Table 1: Pathological Response Rates in the Literature

Trial/Reference	No. of Patients	Agent(s)	No. of Cycles	pCR (%)
Fisher [7]	1523	AC	X 4	9
Buzdar [29]	87	Paclitaxel	X 4	9
Amat [30]	80	Docetaxel	X 6	20
NSABP B27 [20]	1502	AC	X 4	13
Aberdeen [31]	47	CVAP-Docetaxel	X4 and x4	34
Green [24]	258	T-FAC	12 w and x 4	15.7 (q3w Pac) 28.1 (qw Pac)
SWOG0012 [32]	265	AC-Pac	X 4 and 12 w	17%

AC = doxorubicin and cyclophosphamide; CVAP = cyclophosphamide, vincristine, doxorubicin, and prednisone; FAC = Fluorouracil, doxorubicin, and cyclophosphamide; T = Paclitaxel.

2.6. Primary Systemic Therapy in Operable Breast Cancer

The Breast Medical Oncology Department at MD Anderson has been one of the pioneers in the use of neoadjuvant chemotherapy for the treatment of breast cancer. The sequential or concurrent administration of taxane and anthracycline constitute the backbone of early breast cancer treatment. At MD Anderson Cancer Center our current standard of care for the treatment of breast cancer is the sequential administration of paclitaxel 80 mg/m2 IV, weekly for 12 cycles followed by FAC/FEC-regimen, IV, every 3 weeks for 4 cycles. [15] This treatment is administered for a total of 24 weeks. Several studies randomized trials has been conducted in our institution incorporating novel drugs and compared with the standard regimen.
PST has several potential advantages compared with the traditional strategy of surgery followed by adjuvant chemotherapy. In addition, PST reduces the size of the primary tumor and lymph node metastasis in greater than 80% of cases, increasing the probability that breast-conserving surgery can be performed. [13-16] A second advantage of this sequencing schedule is that it permits the assessment of response of the primary tumor to the particular chemotherapy regimen. This assessment allows the opportunity to “cross-over” to a different regimen for an individual patient if there is minimal or no response to the first regimen. These and other theoretic advantages for PST must be balanced carefully with other aspects of individual patient management.
Most of the reports of combined-modality treatment for LABC were based on treatment programs that included anthracycline-containing combination of chemotherapy regimens, such as

fluorouracil, doxorubicin, and Cyclophosphamide (FAC) or FEC when the anthracycline used was Epirubicin. Since the introduction of taxanes over the past decade, there have been several reports in which an anthracycline and taxane combination was utilized.

One of the first considerations for studying PST for breast carcinoma was to investigate whether earlier delivery of chemotherapy offered the possibility of improved survival in patients with locally advanced breast carcinoma. To test these concepts, the National Surgical Adjuvant Breast and Bowel Project (NSABP) began the B-18 trial to test whether sequencing chemotherapy before surgery would improve outcomes. [13-15] The trial enrolled 1523 patients with early-stage, operable breast carcinoma and randomized them to receive four cycles of doxorubicin/Cyclophosphamide (AC) either before or after surgical treatment. The primary end points of this trial were disease-free survival (DFS) and overall survival (OS). With respect to these end points, the trial was a negative study. After 9 years, the OS and DFS were nearly identical between the two groups (P = .80, P =0.5, respectively). A second large randomized prospective trial that directly compared the sequencing of chemotherapy and surgery was performed by the European Organization of Research and Treatment of Cancer (EORTC). [16] This trial randomized 698 patients to preoperative or postoperative chemotherapy comprised of four cycles of FEC (5-Fluorouracil, Epirubicin, and Cyclophosphamide). Like the NSABP B-18 trial, the EORTC study demonstrated equivalent survival and rates of distant metastases between the two treatment arms.

Gianni el al, [17] randomized 1,355 patients with breast cancer > 2 cm to three groups: adjuvant doxorubicin (A) followed by cyclophosphamide, methotrexate, and 5-FU (CMF) (Sx→A→CMF); adjuvant doxorubicin and paclitaxel (AT) followed by CMF (Sx→AT→CMF); and neoadjuvant AT followed by CMF (AT→CMF→Sx). pCR rates in the neoadjuvant arm were 23% in breast only and 20% in breast plus axilla patients. The breast conservative treatment rate was also better in this arm (65% vs. 34%; P <.001). At 5 years of follow up, adjuvant chemotherapy was similar to PST in terms of freedom for progression (P =.24) and OS (P = .81)

A recent meta-analysis addressed directly the question of neoadjuvant versus adjuvant chemotherapy. [18] Nine randomized clinical trials involving 3,946 patients were included. pCR rates were highly variables among these trials. Six trials had a higher rate of BCT after PTS. No difference was observed between the two arms for death, disease progression, or distant recurrence. Surprisingly, PST was associated with a higher locoregional recurrence (risk ratio, [RR] 1.22; P = .15). This greater risk was largely attributed to those trials in which radiation alone without surgery was used in patients who achieved a clinical complete response to PST. (RR, 1.53; P =.009).

2.7. Role of the Pathological Complete Response in Breast Cancer

A pathological complete response (pCR) implies the absence of residual invasive disease following PST. Pathological complete response is associated with long-term survival, and has been adopted as the primary end point for neoadjuvant trials. While it is generally held that a definition of pCR should include patients without residual invasive carcinoma in the breast (pT0), the presence of nodal metastasis, minimal residual cellularity, and residual *in situ* carcinoma are not consistently defined as pCR or residual disease (RD). When there is no residual invasive cancer in the breast, the number of involved axillary lymph nodes is inversely related to survival. [19] Conversely, patients who convert to node-negative status after treatment have excellent survival, even if there is RD in the breast. [20] Symmans and collaborators, [33]

recently introduced a residual breast cancer burden (RCB) index as a novel independent new risk factor that improves the prediction of distant relapse after PST compared with currently used risk factors. RBC can be divided in four categories: patients with minimal residual disease (RCB-I) have the same 5-year prognosis as those with pCR (RCB-0), irrespective of the type of neoadjuvant chemotherapy administered, adjuvant hormonotherapy, or the pathologic stage of RD. Extensive RD (RCB-III) was associated with poor prognosis, irrespective of the type of neoadjuvant chemotherapy administered, adjuvant hormontherapy or the pathologic stage of RCB.

2.8. Primary Systemic Therapy: Sequential versus Alternating Regimens

Several important observation regarding the sequence of anthracycline and taxane-based treatment during PST have raised the question which sequence of treatment will be most favorable to impact in the pCR rates and outcome (DFS and OS) of breast cancer patients. Anthracyclines are usually administered before taxanes (or combined with taxanes), a practice which reflects the sequence of administration used in clinical trials rather than research-based evidence.

Miller et al [34] published in 2005 a phase II randomized biomarker discovery study in patients with operable breast cancer. Seventy patients were treated with the dose dense sequence doxorubicin (75 mg/m2 every 2 weeks, 3 cycles) followed by docetaxel (40 mg/m2 weekly, 6 cycles) (A→D) or the vice versa sequence D→A. Patients treated with D→A had a pCR rate 17% compared with the sequence of A→D with a pCR rate of 8.6%. In the sequence D→A the relative dose intensity (RDI) for A and D were 0.94 and 0.97, respectively. The sequence A→D resulted in RDI for A and D were 0.95 and 0.85, respectively.

The Neo-tAnGo is a large randomized phase III study conducted in United Kingdom, comparing epirubicin plus cyclophosphamide (E/C) followed by paclitaxel with and without gemcitabine compared with paclitaxel with or without gemcitabine followed by epirubicin plus cyclophosphamide. (E/C →Pac+/-Gem vs. Pac+/-Gem →EC). [35] A total of 831 patients with T2 tumors or above, were randomized to a 2-by-2 factorial design, and the primary endpoint of the study was the pCR rate in the breast as well in the axillary lymph nodes. Patient characteristics were well balanced in both arms. The pCR rates were identical for EC → T (17%) compared with the EC → T+Gem sequence (*P* = .98). However, the sequence T+/- Gem → EC, showed a pCR of 20% compared with 15% for the EC then T+/-Gem (P = .03). Adjustment by stratification did not alter the results.

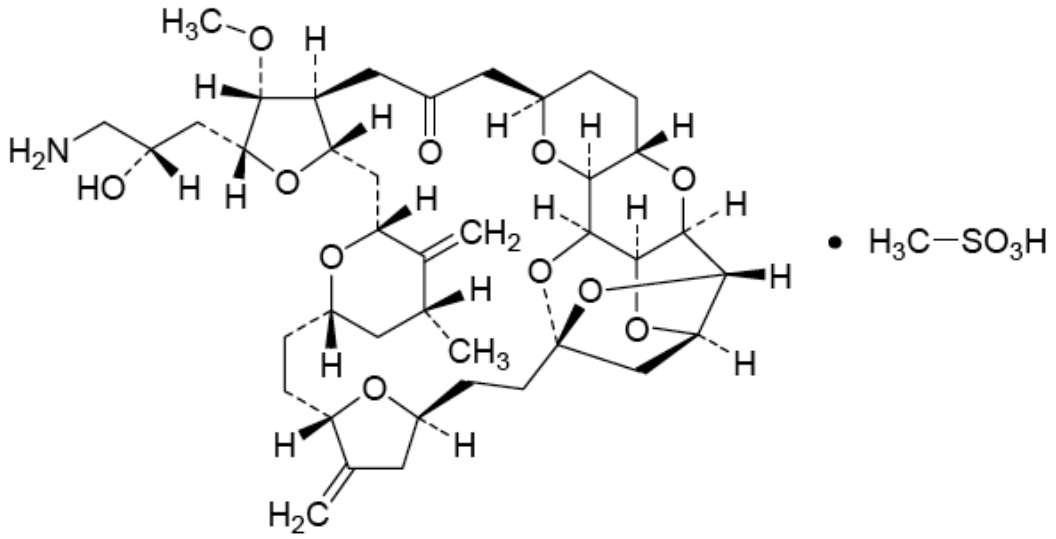
Similarly, Alvarez et al [36]analyzed retrospectively the sequence of chemotherapy with taxanes and anthracyclines in 1,414 patients that received PST at MD Anderson Cancer Center between 1991 and 2009. A total of 1188 patients (84%) received the sequence taxane followed by anthracycline. (T→A) and 226 patients (16%) received the sequence of anthracycline followed by taxane (A→T). A total of 249 patients (21%) achieved a pCR in the T→A sequence compared with 28 patients (12.3%) in the A→T sequence. (*P* = .004). In addition, for all patients that were treated with the sequence T→A the 5-year and 10-year DFS rates were 78.9% and 61.4%, respectively. (*P* = .0001) For patients treated with the sequence A→T, the 5-year and 10-year DFS rates were 57.5% and 45.2%, respectively. (*P* = .0001).

These three studies demonstrated evidence that dose delivery and efficacy improve when taxane is given first with less immediate toxicity. Several ongoing randomized adjuvant trials, such as SOLD and NSABP-B40, taxanes are now given before anthracyclines.

3. ERIBULIN (HALAVEN®)

3.1 Description

Eribulin mesylate (Halaven®) is a non-taxane microtubule dynamics inhibitor. Eribulin is a synthetic analogue of halichondrin B, a product isolated from the marine sponge *Halichondria okadai.* The chemical name for eribulin mesylate is 11,15:21:24,28-Triepoxy-7,9-ethano-12.15-methano-9H,15-furo[3,2-i]furo[2',3':5,6]pyrano[4,3-b][1,4]dioxacyclopetacosin-5(4H)-one,2-[(2S)-3-amino-2-hydroxypropyl]hexacosahydro-3-methoxy-26-methyl-20,27-bis(methaylene)-,(2R,3R,3aS,7R,8aS,9S,10aR,11S,12R,13aR,13bS,15S,18S,21S,24S,26R,28R,29aS)-methanesulfonnate (salt). It has a molecular weight of 826.0 (729.9 for free base). The empirical formula is C_{40} $H_{59}NO_{11}.CH_4O_3S$. Eribulin mesylate has the following structural formula:



Eribulin is clear, colorless, sterile solution for intravenous administration. Each vial contains 1 mg of eribulin mesylate as a 0.5 mg/mL solution in ethanol: water (5:95)

3.2 Clinical Pharmacology

3.2.1 Mechanism of Action

Eribulin inhibits the growth phase of microtubules without affecting the shortening phase and sequesters tubulin into nonproductive aggregates. Eribulin exerts its effects via a tubulin-based antimitotic mechanism leading to G2/M cell-cycle block, disruption of mitotic spindles, and ultimately, apoptotic cell death after prolonged mitotic blockage.

3.2.2 Pharmacodynamics - Cardiac Electrophysiology

The effects of eribulin on the QTc interval was assessed in an open-label, uncontrolled, multicenter, single-arm dedicated QT trial. A total of 26 patients with solid tumors received 1.4 mg/m2 of eribulin on Days 1 and 8 of a 21-day cycle. A delayed QTc prolongation was observed on Day 8, with no prolongation observed on Day 1. The maximum mean QTcF change from baseline (95% upper confidence interval) was 11.4 (19.5) ms.

3.2.3 Pharmacokinetics

The pharmacokinetics of eribulin is linear with a mean elimination half-life of approximately 40 hours, a mean volume of distribution of 43 L/M2 to 114/m2 and mean clearance of 1.16 L/hr/m2 to 2.42 L/hr/m2 over the dose range of 0.25 mg/m2 to 4.0 mg/m2. The human plasma protein binding of eribulin at concentrations of 100 ng/mL to 1,000 ng/mL ranges from 49% to 65% eribulin exposure after multiple dosing is comparable to that following a single dose. No accumulation of eribulin is observed with weekly administration.

3.3 Metabolism

Unchanged eribulin was the major circulating species in plasma following administration of C-eribulin to patients. Metabolite concentrations represented <0.6% of parent compound, confirming that there are no major human metabolites of eribulin.
Cytochrome P450 3A4 (CYP3A4) negligibly metabolizes eribulin *in vitro*. Eribulin inhibits CYP3A4 activity in human liver microsomes, but it is unlikely that eribulin will substantially increase the plasma level of CYP3A4 substrates. Eribulin shows no induction potential for CYP1A, CYP2C9, CYP2C19, and CYP3A in primary hepatocytes. No significant inhibition of CYP1A2, CYP2C9, CYP2C19, CYP2D6, or CYP2E1 was detected with eribulin concentrations up to 5 μM in pooled human liver microsomes. *In vitro* drug interaction studies indicate that eribulin does not inhibit drugs that are substrates of these enzymes and it is unlikely that eribulin will affect plasma levels of drugs that are substrates of CYP enzymes. Eribulin is a substrate and a week inhibitor of the drug efflux transporter P-gp *in vitro*.

3.4 Elimination

Eribulin is eliminated primarily in feces unchanged. After administration of 14C-eribulin to patients, approximately 82% of the dose was eliminated in feces and 9% in urine. Unchanged eribulin accounted for approximately 88% and 91% of the dose in feces and urine, respectively.

3.5 Effects of Age, Gender, and Race

Based on a population pharmacokinetic analysis with data collected from 340 patients, gender, race, and age do not have a clinically meaningful effect on the PK of eribulin.

3.6 Nonclinical Toxicology

Carcinogenicity studies have not been conducted with eribulin mesylate.
Eribulin mesylate was not mutagenic in *in vitro* bacterial reverse mutation assays (Ames test). Eribulin mesylate was positive in mouse lymphoma mutagenesis assays, and was clastogenic in an *in vivo* rat bone marrow micronucleus assay.
The effects of eribulin on human fertility are unknown. Fertility studies have not been conducted with eribulin mesylate in humans or animals. However, nonclinical findings in repeated-dose dog and rat toxicology studies suggest that male fertility may be compromised by treatment with eribulin mesylate. Rats exhibited testicular toxicity (hypocellularity of seminiferous epithelium with hypospermia/aspermia) following dosing with eribulin mesylate at or above 0.43 times the recommended human dose (mg/m^2) given once weekly for 3 weeks, or at or above 0.21 times the recommended human dose (mg/m^2) given once weekly for 3 out of 5 weeks, repeated for 6 cycles. Testicular toxicity was also observed in dogs given 0.64 times the recommended human dose (mg/m^2) weekly for 3 out of 5 weeks, repeated for 6 cycles.

3.7 Clinical Studies

Study 1 was an open-label, randomized, multicenter trial of 762 patients with metastatic breast cancer who received at least two chemotherapeutic regimens for the treatment of metastatic disease and experienced disease progression within 6 months of their last chemotherapeutic regimen. [1] Patients were required to receive prior anthracycline- and taxane- based chemotherapy for adjuvant or metastatic disease. Patients were randomized (2:1) to receive eribulin (n=508) or a single agent therapy selected prior to randomization (control arm, n=254). Randomization was stratified by geographic region, HER2/*neu* status, and prior capecitabine exposure. Eribulin was administered at a dose of 1.4 mg/m^2 on Days 1 and 8 of a 21-dayy. Eribulin-treated patients received a median of 5 cycles (range: 1 to 23 cycles) of therapy. Control arm therapy consisted of 97% chemotherapy (25% vinorelbine, 18% gemcitabine, 18% capecitabine, 16% taxane, 9% anthracycline, 10% other chemotherapy), or 3% hormonal therapy. The main efficacy outcome was overall survival.

Patients demographic and baseline characteristics were comparable between the treatment arms. The median age was 55 (range: 27 to 85 years) and 92% were White. Sixty-four percent of the patients were enrolled in North America/Western Europe/Australia, 25% in Eastern Europe/Russia, and 11% in Latin America/South Africa. Ninety-one percent of patients have a baseline ECOG performance status of 0 or 1. Tumor prognostic characteristics, including estrogen receptor status (positive: 67%, negative: 28%), progesterone receptor status (positive: 49%, negative 39%), HER2/*neu* receptor status (positive: 16%, negative: 74%), triple negative status (ER$^-$, PR$^-$, HER2/*neu*$^-$: 19%), presence of visceral disease (82%, including 60% liver and 38% lung) and bone disease (61%) and number of sites of metastases (greater than two: 50%), were also similar in the eribulin and control arms. Patients received a median of four prior chemotherapy regimens in both arms.

In Study 1, a statistically significant improvement in overall survival was observed in patients randomized to the eribulin arm compared to the control arm (see Table 2). An updated unplanned survival analysis, conducted when 77% of events had been observed (see Figure 1) was consistent with the primary analysis. In patients randomized to eribulin, the objective response rate by the RECIST criteria was 11% (95% CI: 8.6%, 14.3%) and the median response duration was 4.2 months (95% CI: 3.8, 5.0 months).

Table 2: Comparison of Overall SURVIVAL in Eribulin and Control arm – Study 1

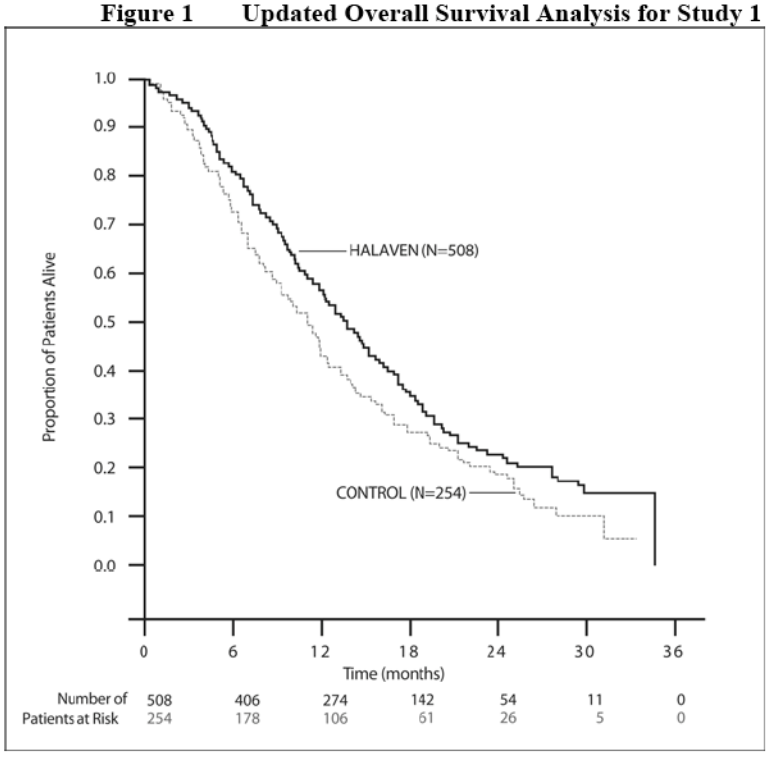
Overall Survival	Eribulin (n=508)	Control arm (n=254)
Primary survival analysis		
Number of deaths	274	148
Median, months (95% CI)	13.1 (11.8, 14.3)	10.6 (9.3, 12.5)
Hazard Ratio (95% CI)[a]	0.81 (0.66, 0.99)	
P value[b]	0.041	
Updated survival analysis		
Number of deaths	386	203
Median, months (95% CI)	13.2 (12.1, 14.4)	10.6 (9.2, 12.0)

CI = confidence interval

[a] Based on Cox proportional hazards model stratified by geographic region, HER2 status, and prior capecitabine therapy.

[b] Based on a log-rank test stratified by geographic region, HER2 status, and prior capecitabine therapy.

Figure 1 Updated Overall Survival Analysis for Study 1



3.8 Drug Supply, Storage and Handling

NDC 62856-389-01 Eribulin mesylate injection, 1mg/2 mL, in a single-use vial. One vial per carton.
Store at 25ºC (77ºF); excursions permitted to 15º - 30ºC (59º - 86ºF). Do not freeze. Store the vials in their original cartons

3.9 Indication and Usage

Eribulin is a microtubule inhibitor indicated for the treatment of patients with metastatic breast cancer who have previously received at least two chemotherapeutic regimens for the treatment of metastatic disease. Prior therapy should have included an anthracycline and a taxane in either the adjuvant or metastatic setting.

3.10 Warning and Precautions

3.10.1 Neutropenia

Severe neutropenia (ANC < 500/mm3) lasting more than one week occurred in 12% (62/503) of patients in Study 1, leading to discontinuation in <1% of patients [see Adverse Reactions (6)]. Patients with alanine aminotransferase or aspartate aminotransferase > 3 × ULN (upper limit of normal) experienced a higher incidence of Grade 4 neutropenia and febrile neutropenia than patients with normal aminotransferase levels. Patients with bilirubin > 1.5 × ULN also had a higher incidence of Grade 4 neutropenia and febrile neutropenia. Monitor complete blood counts prior to each dose; increase the frequency of monitoring in patients who develop Grade 3 or 4 cytopenias. Delay administration of Eribulin and reduce subsequent doses in patients who experience febrile neutropenia or Grade 4 neutropenia lasting longer than 7 days. Clinical studies of Eribulin did not include patients with baseline neutrophil counts below 1,500/mm3.

3.10.2 Peripheral Neuropathy

Grade 3 peripheral neuropathy occurred in 8% (40/503) of patients, and Grade 4 in 0.4% (2/503) of patients in Study 1. Peripheral neuropathy was the most common toxicity leading to discontinuation of Eribulin (5% of patients; 24/503). Neuropathy lasting more than one year occurred in 5% (26/503) of patients. Twenty-two percent (109/503) of patients developed a new or worsening neuropathy that had not recovered within a median follow-up duration of 269 days (range 25-662 days). Monitor patients closely for signs of peripheral motor and sensory neuropathy. Withhold Eribulin in patients who experience Grade 3 or 4 peripheral neuropathy until resolution to Grade 2 or less.

3.10.3 Embryo-Fetal Toxicity

There are no adequate and well-controlled studies of Eribulin in pregnant women. Eribulin is a microtubule inhibitor; therefore, it is expected to cause fetal harm when administered to a pregnant woman. Embryo-fetal toxicity and teratogenicity occurred in rats that received eribulin mesylate at approximately half of the recommended human dose based on body surface area. If this drug is used during pregnancy, or if a patient becomes pregnant while taking this drug, she should be apprised of the potential hazard to the fetus.

3.10.4 QT Prolongation

In an uncontrolled open-label ECG study in 26 patients, QT prolongation was observed on Day, independent of eribulin concentration, with no QT prolongation observed on Day 1. ECG monitoring is recommended if therapy is initiated in patients with congestive heart failure, bradyarrhythmias, drugs known to prolong the QT interval, including Class Ia and III antiarrhythmics, and electrolyte abnormalities. Correct hypokalemia or hypomagnesemia prior to initiating Eribulin and monitor these electrolytes periodically during therapy. Avoid Eribulin in patients with congenital long QT syndrome.

3.11 Dosage and Administration

3.11.1 Recommended Dose

The recommended dose of Eribulin is 1.4 mg/m2 administered intravenously over 2 to 5 minutes on Days 1 and 8 of a 21-day cycle.
The recommended dose of Eribulin in patients with mild hepatic impairment (Child-Pugh A) is 1.1 mg/m2 administered intravenously over 2 to 5 minutes on Days 1 and 8 of a 21-day cycle.
The recommended dose of Eribulin in patients with moderate hepatic impairment (Child-Pugh B) is 0.7 mg/m2 administered intravenously over 2 to 5 minutes on Days 1 and 8 of a 21-day cycle.
The recommended dose of Eribulin in patients with moderate renal impairment (creatinine clearance of 30-50 mL/min) is 1.1 mg/m2 administered intravenously over 2 to 5 minutes on Days 1 and 8 of a 21-day cycle.

3.11.2 Dose Modification

Assess for peripheral neuropathy and obtain complete blood cell counts prior to each dose.
Recommended dose delays
Do not administer Eribulin on Day 1 or Day 8 for any of the following:

− ANC < 1,000/mm3
− Platelets < 75,000/mm3
− Grade 3 non-hematologic AEs (except constitutional symptoms, such as fatigue, muscle aches, insomnia, dry mouth and sweating; and skin changes such as dry skin, pruritus, and nail changes; and constipation) should have all study treatment immediately interrupted pending investigator determination of whether the AEs are related or not related to study treatment.

- The use of G-CSF (granulocyte colony-stimulating factor) or GM-CSF (granulocyte–macrophage colony-stimulating factor) is recommended for patients who receive Eribulin and at treating physician discretion.
  If toxicities resolve or improve to ≤ Grade 2 severity by Day 8, resume Eribulin at 1.4 mg/m²

The Day 8 dose may be delayed for a maximum of 1 week.
− If toxicities do not resolve or improve to ≤ Grade 2 severity by Day 15, omit the dose.
− If toxicities resolve or improve to ≤ Grade 2 severity by Day 15, administer Eribulin at a reduced dose and initiate the next cycle no sooner than ≥ 2 weeks later.

3.11.3 Recommended dose reductions

If a dose has been delayed for toxicity and toxicities have recovered to Grade 2 severity or less, resume Eribulin at a reduced dose as set out in Table 3.
Do not re-escalate Eribulin dose after it has been reduced.

Eribulin dose calculation for patients with BSA of > 2.0. The research nurse will notify the Principal Investigator who will discuss the appropriate dose with the attending physician prior to treatment.

It is well known that obese patients have a high risk to be overdosed using standard BSA measures, especially for highly myelotoxic drugs like eribulin. Therefore, for patients that are going to be treated with eribulin and their BSA ≥2.0. We are rounding down the BSA to 2. This will allow us to manage her blood toxicity more efficiently.

Table 3. Recommended Dose Eribulin Reductions

Adverse Reaction	**Recommended Dose**
Hematological:	
Grade 3 neutropenia without fever lasting more than 7 days	Maintain the recommended dose of **1.4 mg/m²**
Grade 4 neutropenia lasting more than 7 days	
Grade 3 or 4 neutropenia complicated by fever or infection	
Grade 4 thrombocytopenia	
Grade 3 thrombocytopenia complicated by haemorrahage or requiring blood or platelet transfusion	**1.1 mg/m²**
Non-hematological:	
Any Grade 3 or 4 in the previous cycle	
Reocurrence of the adverse reaction:	
Any Grade 3 or 4 despite reduction to 1.1 mg/m²	**0.7 mg/m²**
Any Grade 3 or 4 despite reduction to 0.7 mg/m²	Consider discontinuation

Toxicities graded in accordance with National Cancer Institute (NCI) Common Terminology Criteria for Adverse

Events (CTCAE) version 4.0.

3.11.4 Instructions for Preparation and Administration

Aseptically withdraw the required amount of Eribulin from the single-use vial and administer undiluted or diluted in 100 mL of 0.9% Sodium Chloride Injection, USP. Do not dilute in or administer through an intravenous line containing solutions with dextrose. Do not administer in the same intravenous line concurrent with the other medicinal products. Store undiluted Eribulin in the syringe for up to 4 hours at room temperature or for up to 24 hours under refrigeration

(40°F or/ 4°C). Store diluted solutions of Eribulin for up to 4 hours at room temperature or up to 24 hours under refrigeration. Discard unused portions of the vial.

3.11.5 Dosage Forms and Strengths
Eribulin Injection, 1 mg/2 mL (0.5 mg/mL).

3.11.6 Contraindications
None.

3.12 Adverse Reactions

The following adverse reactions are discussed in detail in other sections of the labeling:

- Neutropenia
- Peripheral neuropathy
- QT interval prolongation

The most common adverse reactions (≥ 25%) reported in patients receiving Eribulin were neutropenia, anemia, asthenia/fatigue, alopecia, peripheral neuropathy, nausea, and constipation. The most common serious adverse reactions reported in patients receiving Eribulin were febrile neutropenia (4%) and neutropenia (2%). The most common adverse reaction resulting in discontinuation of Eribulin was peripheral neuropathy (5%).

Because clinical trials are conducted under widely varying conditions, the adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in other clinical trials and may not reflect the rates observed in clinical practice.

In clinical trials, Eribulin has been administered to 1,222 patients with multiple tumor types, including 240 patients exposed to Eribulin for 6 months or longer. The majority of the 1,222 patients were women (82%) with a median age of 58 years (range: 26 to 91 years). The racial and ethnic distribution was Caucasian (83%), Black (5%), Asian (2%), and other (5%).

The adverse reactions described in Table 2 were identified in 750 patients treated in Study 1. In Study 1, patients were randomized (2:1) to receive either Eribulin (1.4 mg/m2 on Days 1 and 8 of a 21-day cycle) or single agent treatment chosen by their physician (control group). A total of 503 patients received Eribulin, and 247 patients in the control group received therapy consisting of chemotherapy [total 97% (anthracyclines 10%, capecitabine 18%, gemcitabine 19%, taxanes 15%, vinorelbine 25%, other chemotherapies 10%)] or hormonal therapy (3%). The median duration of exposure was 118 days for patients receiving Eribulin and 63 days for patients receiving control therapy. Table 2 reports the most common adverse reactions occurring in at least 10% of patients in either group.

Adverse Reactions with a Per-Patient Incidence of at Least 10% in Study 1

MedDRA ver 10.0	Eribulin n=503		Control Group n=247	
	All Grades	≥ Grade 3	All Grades	≥ Grade 3
Blood and Lymphatic System Disorders[a]				
Neutropenia	82%	57%	53%	23%
Anemia	58%	2%	55%	4%
Nervous system disorders				
Peripheral neuropathyb	35%	8%	16%	2%
Headache	19%	<1%	12%	<1%
General disorders and administrative site conditions				
Asthenia/Fatigue	54%	10%	40%	11%
Mucosal inflammation	9%	1%	10%	2%
Pyrexia	21%	<1%	13%	<1%
Gastrointestinal disorders				
Constipation	25%	1%	21%	1%
Diarrhea	18%	0	18%	0
Nausea	35%	1%	28%	3%
Vomiting	18%	1%	18%	1%
Musculoskeletal and connective tissue disorders				
Arthralgia/Myalgia	22%	<1%	12%	1%
Back pain	16%	1%	7%	2%
Bone pain	12%	2%	9%	2%
Pain in extremity	11%	1%	10%	1%
Investigations				
Weight decreased	21%	1%	14%	<1%
Metabolism and nutrition disorders				
Anorexia	20%	1%	13%	1%
Respiratory, thoracic, and mediastinal disorders				
Cough	14%	0	9%	0
Dyspnea	16%	4%	13%	4%
Skin and subcutaneous tissue disorders				
Alopecia	45%	NAc	10%	NAc
Infections and Infestations				
Urinary Tract Infection	10%	1%	5%	0

[a] based upon laboratory data.

[b] includes neuropathy peripheral, neuropathy, peripheral motor neuropathy, polyneuropathy, peripheral sensory neuropathy, and paraesthesia.

[c] not applicable; (grading system does not specify > Grade 2 for alopecia). Based

Cytopenias: Grade 3 neutropenia occurred in 28% (143/503) of patients who received Eribulin in Study 1, and 29% (144/503) of patient's experienced Grade 4 neutropenia. Febrile neutropenia occurred in 5% (23/503) of patients; two patients (0.4%) died from complications of febrile neutropenia. Dose reduction due to neutropenia was required in 12% (62/503) of patients and discontinuation was required in <1% of patients. The mean time to nadir was 13 days and the mean time to recovery from severe neutropenia (<500/mm3) was 8 days. Grade 3 or greater thrombocytopenia occurred in 1% (7/503) of patients. G-CSF (granulocyte colony-stimulating factor) or GM-CSF (granulocyte–macrophage colony-stimulating factor) was used in 19% of patients who received Eribulin.

Peripheral Neuropathy: In Study 1, 17 % of enrolled patients had Grade 1 peripheral neuropathy and 3% of patients had Grade 2 peripheral neuropathy at baseline. Dose reduction due to peripheral neuropathy was required by 3% (14/503) of patients who received Eribulin. Four percent (20/503) of patients experienced peripheral motor neuropathy of any grade and 2% (8/503) of patients developed Grade 3 peripheral motor neuropathy.
Liver Function Test Abnormalities: Among patients with Grade 0 or 1 ALT levels at baseline, 18% of Eribulin-treated patients experienced Grade 2 or greater ALT elevation. One Eribulin-treated patient without documented liver metastases had concomitant Grade 2 elevations in bilirubin and ALT; these abnormalities resolved and did not recur with re-exposure to Eribulin.
Less Common Adverse Reactions: The following additional adverse reactions were reported in ≥ 5% to <10% of the Eribulin-treated group:

- **Eye Disorders:** increased lacrimation
- **Gastrointestinal Disorders:** dyspepsia, abdominal pain, stomatitis, dry mouth
- **General Disorders and Administration Site Conditions:** peripheral edema
- **Infections and Infestations:** upper respiratory tract infection
- **Metabolism and Nutrition Disorders:** hypokalemia
- **Musculoskeletal and Connective Tissue Disorders:** muscle spasms, muscular weakness
- **Nervous System Disorders:** dysgeusia, dizziness
- **Psychiatric Disorders:** insomnia, depression
- **Skin and Subcutaneous Tissue Disorders:** rash

To report SUSPECTED ADVERSE REACTIONS, contact Eisai Inc. at (1-877-873-4724) or contact FDA at 1-800-FDA-1088 or www.fda.gov/medwatch

3.13 Drug Interactions

3.13.1 Effects of Other Drugs on Eribulin

No drug-drug interactions are expected with CYP3A4 inhibitors and P-gp inhibitors. The effect of ketoconazole, a strong inhibitor of cytochrome P450 3A4 (CYP3A4) and a P-gp inhibitor, on the pharmacokinetics (PK) of eribulin was studied in an open-label, two-treatment, two-sequence, two-way crossover trial in 12 patients with advanced solid tumors. The mean dose-normalized AUC values were similar when eribulin was administered with or without ketoconazole (ratio of the mean AUC: 0.97; 90% CI: 0.83, 1.12).

3.13.2 Effect of Eribulin on Other Drugs

Eribulin does not inhibit CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1 or CYP3A4 enzymes or induce CYP1A2, CYP2C9, CYP2C19 or CYP3A4 enzymes at relevant clinical concentrations. Eribulin is not expected to alter the plasma concentrations of drugs that are substrates of these enzymes.

3.13.3 Use in Specific Populations

3.13.3.1 Pregnancy Category D

There are no adequate and well-controlled studies with Eribulin in pregnant women. Eribulin is a microtubule inhibitor; therefore, it is expected to cause fetal harm when administered to a pregnant woman. Embryo-fetal toxicity and teratogenicity occurred in rats that received eribulin mesylate at approximately half of the recommended human dose based on body surface area. If this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus.
In a developmental toxicity study, pregnant rats received intravenous infusion of eribulin mesylate during organogenesis (Gestation Days 8, 10, and 12) at doses approximately 0.04, 0.13, 0.43 and 0.64 times the recommended human dose, based on body surface area (mg/m2). Increased abortion and severe external or soft tissue malformations were observed in offspring at doses 0.64 times the recommended human dose based on body surface area (mg/m2), including the absence of a lower jaw, tongue, stomach and spleen. Increased embryo-fetal death/resorption, reduced fetal weights, and minor skeletal anomalies consistent with developmental delay were also reported at or above doses of 0.43 times the recommended human dose.
Maternal toxicity of eribulin mesylate was reported in rats at or above doses of 0.43 times the recommended human dose (mg/m^2), and included enlarged spleen, reduced maternal weight gain and decreased food consumption.

3.13.3.2 Nursing Mothers

It is not known whether Eribulin is excreted into human milk. No studies in humans or animals were conducted to determine if Eribulin is excreted into milk. Because many drugs are excreted into human milk and because of the potential for serious adverse reactions in human milk fed infants from Eribulin, a decision should be made whether to discontinue nursing or to discontinue Eribulin taking into account the importance of the drug to the mother.

3.13.3.3 Pediatric Use

The safety and effectiveness of Eribulin in pediatric patients below the age of 18 years have not been established.

3.13.3.4 Geriatric Use

Study 1 did not include sufficient numbers of subjects aged 65 years and older to determine whether they respond differently from younger subjects. Of the 827 subjects who received the recommended dose and schedule of Eribulin in clinical studies, 15% (121/827) were 65 and older, and 2% (17/827) patients were 75 and older. No overall differences in safety were observed between these subjects and younger subjects.

3.13.4 Hepatic Impairment

A study evaluated the PK of eribulin in patients with mild (Child-Pugh A; n=7) and moderate (Child-Pugh B; n=5) hepatic impairment. Compared to patients with normal hepatic function (n=6), eribulin exposure increased 1.8-fold and 2.5-fold in patients with mild and moderate hepatic impairment, respectively. Administration of Eribulin at a dose of 1.1 mg/m2 to patients with mild hepatic impairment and 0.7 mg/m2 to patients with moderate hepatic impairment

resulted in similar exposure to eribulin as a dose of 1.4 mg/m2 to patients with normal hepatic function. A lower starting dose of 1.1 mg/m2 is recommended for patients with mild hepatic impairment (Child-Pugh A) and of 0.7 mg/m2 is recommended for patients with moderate hepatic impairment (Child-Pugh B). Eribulin was not studied in patients with s severe hepatic impairment (Child-Pugh C).

3.13.5 Renal Impairment

No formal PK trials were conducted with Eribulin in patients with renal impairment. Available data suggests that no dose adjustment is necessary for patients with mild renal impairment (CrCl 50-80 mL/min). However, for patients with moderate renal impairment (CrCl 30-50 mL/min), the geometric mean dose-normalized systemic exposure increased 2-fold compared to patients with normal renal function. A lower starting dose of 1.1 mg/m2 is recommended for patients with moderate renal impairment. The safety of Eribulin was not studied in patients with severe renal impairment (CrCl < 30 mL/min).

3.13.6 Overdosage

Overdosage of Eribulin has been reported at approximately 4 times the recommended dose, which resulted in Grade 3 neutropenia lasting seven days and a Grade 3 hypersensitivity reaction lasting one day. There is no known antidote for Eribulin overdose.

4. TREATMENT PLAN

4.1 Study Design

This is a randomized, multi-center, open-label, Phase II study of sequential eribulin followed by FAC/FEC-regimen or Paclitaxel followed by FAC/FEC-regimen as neoadjuvant therapy in women with operable invasive breast cancer patients whose HER2 is not over-expressed.

Approximately 162 women will be randomly assigned on a 1:1 basis to either arm. 81 randomized patients per arm.

4.2 Eligibility

4.2.1 Inclusion Criteria:

To be included in the study, the subject must have:

1) Signed written informed consent
2) Histologically confirmed primary invasive adenocarcinoma of the breast.
3) Clinical stage breast cancer T2-3, N0-3, M0
4) Negative HER-2/neu expression as determined by local hospital laboratory using Fluorescence In Situ Hybridization (FISH), or is less or equal to 1+ using Immunohistochemistry (IHC).
5) No prior treatment for primary invasive adenocarcinoma of the breast such as irradiation, chemotherapy, hormonal therapy, immunotherapy, investigational therapy or surgery. Subjects receiving hormone replacement treatment (HRT) are eligible if this therapy is discontinued at least 2 weeks before starting study treatment. Treatment for DCIS is allowed, such as surgery, hormonal therapy and radiotherapy.
6) Karnofsky performance status (KPS) of 80 – 100.
7) The ability and willingness to comply with scheduled visits, treatment plan, laboratory tests, and other study procedures.
8) Baseline MUGA or echocardiogram scans with LVEF of > 50%.
9) Normal PTT and either INR or PT < 1.5 x ULN.
10) Men or women 18 years of age or older.
11) Women of childbearing potential (WOCBP) must agree to use a medically acceptable method of contraception to avoid pregnancy throughout the study and for up to 8 weeks after the last dose of study drugs.
12) Willingness to have core biopsies and/or FNA performed before the start of study treatment and at the end of 12 week on treatment.

4.2.2 Exclusion Criteria:

1) Women who are pregnant (including positive pregnancy test at enrollment or prior to study drug administration) or breast-feeding.
2) Disease free of prior malignancy for < 5 years with the exception of DCIS, curatively treated basal carcinoma of the skin, local skin squamous cell carcinoma or carcinoma in situ of the cervix.
3) Absolute neutrophil count (ANC) < 1500/mm3
4) Total bilirubin > 1.5 times the upper limit of normal (ULN)
5) AST or ALT > 2.5 times the upper limit of normal (ULN)
6) Platelets < 100,000/mm3.
7) Serum creatinine > 1.5 x ULN or creatinine clearance < 60 mL/min (measured or calculated by Cockcroft-Galt method)
8) Evidence of metastatic breast cancer following a standard tumor staging work-up.
9) Evidence of inflammatory breast cancer.
10) Evidence of any grade 2 sensory or motor neuropathy.
11) Known human immunodeficiency viral (HIV) infection
12) Serious intercurrent infections or non-malignant medical illness that are uncontrolled or the control of which may be jeopardized by this therapy.
13) Psychiatric disorders or other conditions rendering the subject incapable of complying with the requirements of the protocols.

4.3 Drug Administration Plan

Randomization will be stratified by tumor size at baseline, estrogen and progesterone expression status and Investigator Site.

ARM 1: Patients will receive Paclitaxel 80 mg/m2 IVPB over 1 hour weekly for 12 doses followed by FAC/FEC.

ARM 2: Patients will receive eribulin 1.4 mg/m2 IV infusion or per institutional guidelines over 2-5 minutes on days 1 and 8 every 3 weeks for 4 cycles (21 day cycle) followed by FAC/FEC.

Patients on **both arms** will receive either FEC or FAC x 4 cycles (21 day cycle) at the preference of the treating physicians.

FEC Chemotherapy:

- 5-Flurouracil 500mg/m2 IV on day 1
- Epirubicin 100mg/m2 IV on day 1
- Cyclophosphamide 500mg/m2 IV on day 1

FAC Chemotherapy: (may be given instead of FEC)

- 5-Flurouracil 500mg/m2 IV on day 1
- Doxorubicin 50mg/m2 IV on day 1 over 30 minutes continuous infusion or IV bolus (as per institutional standard)
- Cyclophosphamide 500mg/m2 IV on day 1

Treatment Modification
Patients who progress in the breast while on eribulin or paclitaxel prior to or at completion of the first 12 weeks of treatment will discontinue eribulin or paclitaxel and will automatically be considered non-responders to eribulin or paclitaxel and then will start the FAC/FEC-regimen.

Patients who progress outside of the breast/present with metastatic disease will be taken off study. We will attempt to confirm progression histologically (biopsy).

Patients who discontinue eribulin or paclitaxel prior to 12 weeks due to toxicity will be evaluated for response to FAC/FEC-regimen unless the PI believes that proceeding directly to surgery would be in the best interest of the patient (e.g., subjects with large tumors that progress rapidly on eribulin or paclitaxel.) If progressive disease occurs while on FAC/FEC- treatment, chemotherapy will be discontinued and the subjects should remain on the study until after they have undergone definitive surgery.

4.3.4 Surgery

All patients will undergo definitive breast surgery 4 -6 weeks from last dose of FAC/FEC-regimen. It is up to the surgeon's discretion if tumors must be removed by either lumpectomy with axillary dissection (i.e. breast conservation surgery) or modified radical mastectomy (i.e. mastectomy with axillary clearance) after discussion with the patient. The surgical specimens

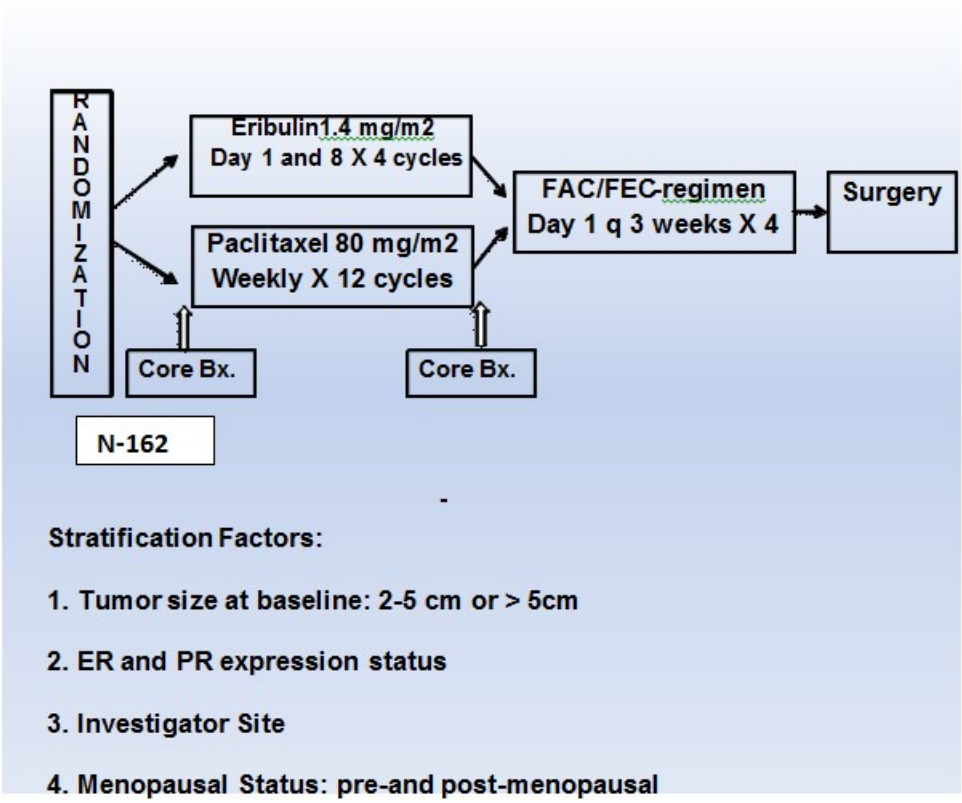
(breast and axillary lymph node tissue) will be evaluated for pathological complete response (defined per protocol) by central Pathologists at M.D. Anderson Cancer Center.

4.4 Duration of Study

The study is expected to be completed within 36 months from study initiation. The duration of chemotherapy treatment per subject will be approximately 6 months, corresponding to 4 cycles of eribulin followed by 4 cycles of FAC/FEC-regimen or 12 cycles of weekly paclitaxel followed by 4 cycles of FAC/FEC-regimen.

This study will be conducted at the University of Texas MD Anderson Cancer Center main campus and the Regional Care Centers (RCC).

Study Schema



4.5 Dose modification

4.5.1 Eribulin modification: please refer to section 3.11.3 (Table 3)

4.5.2 Weekly Paclitaxel

Patient on weekly paclitaxel will continue paclitaxel without dose modification if ANC $\geq$ 1000/mm^3 and Plt $\geq$100,000/mm^3 on day of the next dose.

Table 1. Paclitaxel Dose Modification

Event	Paclitaxel Dose Modification
Neutropenia	

≥ 1000/mm3	No change to paclitaxel.
	• For ANC ≤ 1500/mm3 consider the use of prophylactic myeloid growth factors (filgrastim), Neuopogen®
	  • Start on day 2 and use for 1-2 days according to patient need, at physician discretion, and to avoid dose reduction.
	  • Growth factor should not be given on the same day as chemotherapy.
	Note: Pegfilgrastim Neulasta® may not be used with paclitaxel due to the weekly dosing in this study.

<1000/mm3	Hold paclitaxel until ANC > 1000/mm3. Resume paclitaxel based on timing of recovery: • ≤ 1 week—no change to paclitaxel • Recheck CBC at Physician discretion • 1 but < 3 weeks—reduce paclitaxel dose by 25% for all subsequent cycles • ≥ 3 weeks—stop paclitaxel. Patient should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Patient remains on study for outcome assessment
Neutropenic Fever	
ANC ≤ 1000/mm3, fever ≥ 38.5°C	• Hold paclitaxel until resolved (ANC > 1000/mm3, fever < 38.5°C). Resume paclitaxel according to number of episodes: • First episode: no change in paclitaxel • Second episode: 25% dose reduction of paclitaxel for all subsequent cycles • Third episode: stop paclitaxel. Patient should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Patient remains on study for outcome assessment.

Event	**Paclitaxel Dose Modification**

	If paclitaxel is held for 3 weeks in a row, stop paclitaxel. Patients should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Patient remains on study for outcome assessment. **Note**: GCSF may be used between days 2–6 according to patient need, at physician discretion, and to avoid dose reduction. Pegfilgrastim Neulasta® may not be used with paclitaxel due to the weekly dosing in this study.
Thrombocytopenia	
≥100,000/mm3	No change to paclitaxel.
75–99,999/mm3	Hold paclitaxel until ≥ 100,000/mm3, resume paclitaxel based on timing of recovery: • ≤ 1 week—no change to paclitaxel. • 1 but < 3 weeks—reduce paclitaxel dose by 25% for all subsequent cycles. • ≥ 3 weeks—stop paclitaxel. Patient should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Patient remains on study for outcome assessment.
< 75,000/mm3	Hold paclitaxel until ≥ 100,000/mm3. Resume paclitaxel with a 25% dose reduction for all subsequent cycles. If paclitaxel is held for 3 weeks in a row, stop paclitaxel. Patient should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Patient remains on study for outcome assessment.
Anemia	
All Grades	No change in paclitaxel. For all anemia events related to paclitaxel regardless of grade, iron studies should be checked and iron should be replaced as indicated. • Red blood cell transfusions can be given at the investigators' discretion as needed for symptom control.

Hepatic	
Grade 0 or 1	No change in paclitaxel

Grade 2	<u>Grade 2 bilirubin:</u> Hold paclitaxel until bilirubin resolves to ≤ grade 1. Resume paclitaxel based on time of recovery. • If bilirubin resolves to ≤ grade 1 in < 2 weeks, resume paclitaxel at previous dose. • If bilirubin remains at grade 2 after holding two consecutive doses of paclitaxel (2 weeks), resume paclitaxel with a 25% reduction in dose for all subsequent doses. • If paclitaxel is held for 3 weeks in a row, stop paclitaxel. Patient should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Patient remains on study for outcome assessment. A rise in indirect bilirubin with a normal direct bilirubin believed to be attributable to Gilbert's disease does not require change in dose or a drug hold. A note to file should be created. **<u>Grade 2 AST or ALT:</u>** Hold paclitaxel until AST/ALT resolve to ≤ grade 1. • If AST/ALT resolve to ≤ grade 1 in < 3 weeks, resume paclitaxel at previous dose. • If paclitaxel is held for 3 weeks in a row, stop paclitaxel. Patient should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Patient remains on study for outcome assessment
Grade 3	<u>Grade 3 bilirubin (not due to Gilbert's disease):</u> <u>Stop paclitaxel</u>. Patient should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Patient remains on study for outcome assessment. <u>Grade 3 AST or ALT:</u> Hold paclitaxel until AST/ALT resolve to ≤ grade 3. Resume paclitaxel at the previous dose. • If AST/ALT remains at grade 3 after holding two consecutive doses of paclitaxel, resume paclitaxel with a 25% dose reduction for all subsequent doses. • If paclitaxel is held for 3 weeks in a row, stop paclitaxel. Patient should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Patient remains on study for outcome assessment.

Grade 4	Grade 4 bilirubin, AST or ALT: Stop paclitaxel. Patient should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Patient remains on study for outcome assessment.
Nausea/Vomiting	
Grade 0–2	No change to paclitaxel.
≥ Grade 3	Hold paclitaxel until resolved to ≤ grade 1. • Resume paclitaxel at previous dose with modification of pre-medications. • For second episode ≥ grade 3 despite maximal supportive care: ➢ Resume paclitaxel with a 25% dose reduction for all subsequent doses
Mucositis	
Grade 0-2	No change in paclitaxel.
≥ Grade 3	Hold paclitaxel until resolved to ≤ grade 1. • Resume paclitaxel at the previous dose, with modification of premedications. • For second episode ≥ grade 3 despite maximal supportive care: ➢ Resume paclitaxel with a 25% dose reduction for all subsequent cycles.
Neurotoxicity	
Grade 0–1	No change to paclitaxel.
Grade 2-3	Hold paclitaxel until neuropathy improves to ≤ grade 1. • Resume paclitaxel with a 25% dose reduction for all subsequent cycles. If paclitaxel is held for 3 weeks in a row for neuropathy, stop paclitaxel. Patient should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Patient remains on study for outcome assessment.
Grade 4	Stop paclitaxel. Patient should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Patient remains on study for outcome assessment
Anaphylaxis/Hypersensitivity	

Mild (*e.g.*, mild flushing, rash, pruritis)	Complete paclitaxel infusion. • No treatment required, but observe patient at least until symptoms have resolved.
Moderate (*e.g.*, moderate flushing, rash, mild dyspnea, chest discomfort)	Stop paclitaxel infusion. • Give intravenous diphenhydramine 20–25 mg and intravenous dexamethasone 10 mg. If symptoms resolve: • Resume paclitaxel infusion after recovery of symptoms at half the previous rate for 15 minutes. If no recurrence of symptoms, the planned rate may be resumed. If symptoms recur after paclitaxel re-challenge: • Stop paclitaxel infusion and stop all subsequent paclitaxel therapy. Patient should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Patient remains on study for outcome assessment.
Severe (*e.g.*, hypotension requiring pressors, angioedema, respiratory distress requiring bronchodilators)	Stop paclitaxel infusion. • Administer diphenhydramine 25 mg and dexamethasone 10 mg IV. Add epinephrine or bronchodilators as needed per institutional guidelines. • Stop all subsequent paclitaxel therapy. Patient should proceed with additional chemotherapy or surgery at the discretion of the treating physician. Patient remains on study for outcome assessment.
Other Clinically Significant Toxicity Excluding Fatigue, Alopecia, and Leukopenia at Physician Discretion	
Grade 0 or 1	No change to paclitaxel.
Grade 2	Hold paclitaxel until resolved to ≤ grade 1. Resume paclitaxel at previous dose. • Increase supportive care measures if possible.
≥ Grade 3	Hold paclitaxel and contact the DCC for further instruction (1-877-303-0226). If ≥ grade 3toxicity recurs, • Stop paclitaxel and contact the DCC for further instruction (1-877-303-0226).

Dose modification for FEC/FAC is based on standard practice per treating physician.

4.5.4 Dose adjustments in hepatic impairment

Alkaline Phosphatase		AST +/or ALT	Dose
<2.5 x ULN	and	<1.5 x ULN	100%
2.5 – 5 x ULN	and	1.6 – 5 x ULN	75%
>5 x ULN	and	>5 ULN	Hold Treatment

4.5.5 Dosage in grade 3/4 cutaneous reactions

Reduce by 20%; further reduce by 20% if reactions continue.

4.5.6 Dose adjustments in myelosuppression

No dose adjustment necessary. Up to 7 days therapy delay allowed if ANC < 1,000. The use of G-CSF support is permitted after the first cycle of therapy. If a delay of therapy > 7days occurs, G-CSF will be used with the next cycle with peg-filgrastin 6 mg SC 24 hours after FEC (or FAC).

4.5.7 Dose adjustments in renal impairment

No dose adjustment necessary.

4.6 Evaluation during Study

See Study Calendar for evaluations during Study.

4.7 Concurrent and supportive care

In general, the use of any concomitant medication/therapies deemed necessary for the care of the patient are allowed, including drugs given prophylactically (eg, Antiemetics +/- steroids, colony stimulating factors), with the following exceptions:

- No other investigational therapy should be given to patients.
- No anticancer agents other than the study medications administered as part of this study protocol should be given to patients.

The concurrent use of all other drugs, over-the-counter medications, or alternative therapies including herbal supplements, specifically, St. John's Wort must be documented in the medical record

4.7.1 Eribulin: No premedication needed.

Medications to avoid with eribulin therapy that may cause QTc prolongation: Appendix D.

4.7.2 Paclitaxel Premedication:

Dexamethasone 10 mg in NS 50 mL IV, 30 minutes prior to weekly paclitaxel infusion. Taper dexamethasone to 4 mg IV after the 3rd dose if no infusion related reaction was noted previously.

Compazine 10 mg PO every 6 hours PRN per nausea is recommended.

4.7.3. FAC or FEC-regimen Premedication:

On Day 1 of each Cycle 30 minutes prior to chemotherapy:

a) Ondansetron 8 mg IV,
b) Lorazepam 1 mg IV,
c) Dexamethasone 20 mg IV.

After chemotherapy infusion:
a) Ondansetron 8 mg PO every 8 hours during first 48 hours,
b) Compazine 10 mg PO every 6 hours PRN per nausea.

4.8 Criteria for removal from the study

Treatment will continue until one of the following criteria is met:

- Patient withdrew consent
- Completion of all prescribed protocol therapy.
- In the judgment of the investigator the continuation of study treatment is not in the best interests of the patients.

4.9 Criteria for Response

The primary endpoint of this study is to assess pathologic complete response (pCR) will be determined based on the routine clinical pathology report. Response categories will be assigned as follows:
Pathologic complete response (pCR) is defined in this study as complete absence of any viable invasive cancer cells in the resected breast and lymph nodes.

4.9.1 Definition of Residual Cancer Burden

We will measure the residual cancer burden (RCB) as a continuous variable derived from the primary tumor dimensions, cellularity of the tumor bed, and axillary nodal burden. RCB can also divided in four classes (RCB-0 to RCB-III)
RCB-0 (pCR), Minimal RCB (RCB-I), Moderate RCB (RCB-II), and Extensive RCB (RCB-III)
The following parameters are required from pathologic examination in order to calculate Residual Cancer Burden (RCB) after neoadjuvant treatment:

1. The largest two dimensions (mms) of the residual tumor bed in the breast (largest tumor bed if multicentric disease)
2. Submission of the entire largest cross-sectional area of the residual tumor bed for histologic mapping, with specific identification of those slides in the pathology report (e.g. "the largest cross-sectional area of primary tumor bed was submitted in cassettes A5 - A9")
   - If the residual tumor is large (i.e. largest diameter > 5 cm), then at least 5 representative cassettes from the largest cross-sectional area are sufficient, but should be identified in the original pathology report (e.g. "representative sections from the largest cross-sectional area of primary tumor bed were submitted in cassettes A5 - A9")
3. Histologic assessment of the percentage of the tumor bed area that contains carcinoma (all carcinoma, i.e. invasive and in situ), select one of the following:
   0%, 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%
   - To assess cellularity it is helpful to scan across the sections of tumor bed and then estimate the average cellularity from the different microscopic fields.
   - When estimating percentage cancer cellularity in any microscopic field, compare the involved area with obvious standards, e.g. more or less than half, one quarter, one fifth, one tenth, one twentieth, etc.
   - Expect there to be variable cellularity within the cross section of any tumor bed, but estimate the overall cellularity from the average of the estimates in different microscopic fields of the tumor bed.
   - e.g. if cellularity in different fields of the tumor bed were estimated as 20%, 10%, 20%, 0%, 20%, 30%, then an average estimate of overall cellularity would be 20%.
4. Histologic estimate of the percentage of the carcinoma in the tumor bed that is in situ, select one of the following:
   0%, 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%
5. The number of positive (metastatic) lymph nodes
6. The largest diameter (mm) of the largest nodal metastasis

The RCB can be access online: www.mdanderson.org/breastcancer_RCB

5. CORRELATIVE STUDIES

Research Hypothesis: Sequential administration of eribulin followed by FAC/FEC-regimen, has greater activity based on pCR rate to the historical sequential administration of paclitaxel followed by FAC/FEC-regimen as neoadjuvant therapy for women with early breast cancer with tumors that do not overexpress HER-2.

5.1 Hot Spot Mutation Analysis Methodology: DNA will be extracted using the QiaAMP microkit (Qiagen Inc. Valencia, CA) according to manufacturer's instructions. A mass spectroscopy-based approach evaluating single nucleotide polymorphisms (SNPs) will be used to detect known mutations in *PIK3CA, AKT1, AKT2, AKT3, PHLPP2,* mTOR, *Rictor, PDPK1, MC1R, BRAF, HRAS, KRAS, MEK1, MEK2, NRAS, RAF1, PRKAG1, GNAC, EGFR, FGFR1, FGFR2, FGFR3, KIT, VGFR, ER, MET, ALK, GNAS, CDK4, CDKN2A, CTNNB1, FBXW7, JAK2, RET, FLT3*. Polymerase chain reaction (PCR) and extension primers for each gene will be designed using Sequenom, Inc. (San Diego, CA) Assay Design. PCR-amplified DNA will be cleaned using EXO-SAP (Sequenom) primer extended by IPLEX chemistry, desalted using Clean Resin (Sequenom) and spotted onto Spectrochip matrix chips using a nanodispenser (Samsung). Chips will be run in duplicate on a Sequenom MassArray MALDI-TOF MassArray system. Sequenom Typer Software and visual inspection will be used to interpret mass spectra. Reactions where more than 15% of the resultant mass runs in the mutant site in both reactions will be scored as positive.

5.2 Molecular Inversion Probes (MIP) Arrays Methodology: The MIP assay has been described, [37] including a study conducted by our group on samples from the SPORE population. DNA is extracted from tumor material that has at least 80% tumor cells based on review by a dedicated breast cancer pathologist by repeat lysis using a Qiagen cocktail (Qiagen, Valencia, CA). MIP probes are oligonucleotides with two complementary end sequences to two adjacent genomic sequences, such that the ends anneal to the DNA in an inverted fashion with a single 'detector' base between. The 'detector' can be either the site of a germline SNP or a somatic mutation that occurs as a single nucleotide change. The MIP probe is hybridized to genomic DNA and split into two tubes that contain paired fluorescent nucleotide mixes (triphosphates of A+T or C+G). In the presence of polymerase and ligase, the MIP probe circularizes with the complementary nucleotide. An important advantage of the MIP technology is that allelic discrimination is enzymatically derived, fluorescent, and highly specific, allowing for multiplexed assays (presently 330,000 probes) with very precise quantization of signals. There is no "bleed through" of a second allele, as often happens with the differential hybridization in standard SNP arrays. Critical for small samples such as biopsies and FFPE (where it is challenging if not impossible to perform robust whole genome amplification), MIP arrays provide accurate results with only 37 ng of input DNA, which is an order of magnitude lower than that required for SNP arrays.

5.3 CTCs blood collection: Cytokines and Micro RNA.A 10-mL red-top Vaccutainer tube will be collected to measure serum levels of soluble markers using Millipore 37-Plex Luminex multiplex assay. Additionally, we will isolate total mRNA for the detection of micro RNAs by RT-PCR. The miRNA to be assessed are miR-221, miR-222, miR-17-5p, miR-17-3p, miR-18a, miR-19a, miR-20a, miR-19b, miR-92-1, let-7f and miR-27b, U6 (control).

CTC measurement: 5 ml of peripheral blood will be collected in AdnaCollect tubes and transported to the lab on ice for processing. We will measure CTC using the PCR based AdnaTest Breast Detect assay.

EMT CTC: 7 ml of peripheral blood in EDTA (purple top Vaccutainer) will be collected for the isolation of peripheral blood mononuclear cells and subsequently depletion of

CD326+/EpCAM+ cells and peripheral blood CD45+ leukocytes, isolation of RNA and detection of EMT-inducing transcription factor transcripts by RT-PCR.

Patients agreeing to participate in optional procedures will have the correlative samples obtained when logistically feasible. Optional procedures not obtained will not be considered protocol deviations.

6. STUDY DRUG COMPLIANCE AND ACCOUNTABILITY

6.1 Assessment of Accountability

The investigator, or an approved representative, e.g. pharmacist, will ensure that all investigational products are stored in a secured area, under recommended storage conditions and in accordance with applicable regulatory requirements. All study drug supplies must be kept in a locked limited access room. The study drug must not be used outside the context of the protocol. Under no circumstances should the investigator or other site personnel supply study drug to other investigators, patients, or clinics, or allow supplies to be used other than directed by this protocol.

7. Adverse Event Reporting and data collection

7.1 Adverse Event Reporting

An adverse event (AE) is any condition that appears or worsens after the subject is enrolled in an investigational study. AE's will be graded by numerical score according to the NCI Common Terminology Criteria Adverse Events (CTCAE) version 4.0 (http://ctep.cancer. gov/forms/CTCAEv4. pdf). Adverse Events not included in the defined NCI CTCAE should be scored according to their impact on the subject's ability to perform daily activities as follows:

- Mild: no limitations to normal activities
- Moderate: causes some limitations to normal activities
- Severe: causes inability to carry out usual activities of daily living Reporting Requirements for Baseline Adverse Events

Although a pertinent positive finding identified on baseline assessment is not an AE, when possible it is to be documented as 'Course Zero' using CTC/CTCAE terminology and grade. An expedited AE report is not required if a patient is entered on a protocol with a pre-existing condition (e.g., elevated laboratory value, diarrhea). The baseline AE must be re-assessed throughout the trial and reported if it fulfills expedited AE reporting guidelines.

- If the pre-existing condition worsens in severity, the investigator must reassess the event to determine if an expedited report is required.
- If the AE resolves and then recurs, the investigator must re-assess the event to determine if an expedited report is required. No modification in grading is to be made to account for abnormalities existing at baseline.

7.2 Expedited Adverse Event Reporting Guidelines

Any AE falling under the definition of serious requires submission of a written report to the Institutional Review Board (IRB) via the Office of Protocol Research (OPR). SAEs will be required to be reported from the time that consent is signed, during the course of treatment and

within 30 days after the last day of active treatment. Beyond 30 days of treatment, completion of only those SAEs that, in the judgment of the investigator, are definitely, possibly or probably related to the study treatment will required to be reported. All SAEs should be reported:
Within 24 hours from the time the PI becomes aware of the event: All events resulting in a participant's death.
Within 5 working days from the time the PI becomes aware of the event: All serious AEs other than that stated in point #1.

7.3 Serious Adverse Event (SAE) Reporting to Eisai

Within 24 hours of first awareness of the event (immediately if the event is fatal or life-threatening), Principal Investigator will report to the Eisai by facsimile any Serious Adverse Event ("SAE," as defined below) that occurs during the SAE reporting period (as defined below) in a Study subject assigned to receive the Eisai Product. Principal Investigator will report such SAEs using an FDA MEDWATCH form and the Serious Adverse Event Fax Cover Sheet provided by Eisai. SAEs should be reported as soon as they are determined to meet the definition, even if complete information is not yet available.

7.4 SAE Definition

A serious adverse event is defined by ICH Guideline E2A and Federal Regulation 62, Oct. 7, 1997 as those events, occurring at any dose, which meets any of the following criteria:

- Results in death,
- Is immediately life-threatening,
- Requires inpatient hospitalization or prolongation of existing hospitalization.
- Results in persistent or significant disability/incapacity,
- Is a congenital abnormality/birth defect
- Any other medical event that, in the medical judgment of the Principal Investigator, may jeopardize the subject or may require medical or surgical intervention to prevent one of the outcomes listed above is also considered an SAE. A planned medical or surgical procedure is not, in itself, an SAE.
- In addition, events that may not meet these criteria, but which the investigator finds very unusual and/or potentially serious, will also be reported in the same manner. All SAEs that have not resolved by the end of the study, or that have not resolved upon discontinuation of the patient's participation in the study, must be followed until either:
- The event resolves, or
- The event stabilizes, or
- The event returned to baseline if a baseline value is available, or
- The event can be attributed to other than the study drug or other than study conduct.

7.5 Adverse Event Data Collection

The following information will be collected for all adverse events:

- Start and stop dates
- Severity (grade)
- Relationship to study drug (attribution)

Whether or not the subject discontinued treatment due to the AE
Note all AEs on the Adverse Event Case Report Form (CRF) whether or not related to study drug.

AE grading and assignment of attribution require documentation by medical personnel who are directly involved in the clinical care of protocol subjects.

7.6 AE/SAE Follow up

All AEs/SAEs, including laboratory abnormalities, that in the opinion of the investigator are clinically significant, will be followed up according to good medical practices.

NOTE: *If a subject begins a new anticancer therapy, the adverse event reporting period for non-serious adverse events ends at the time the new treatment is started. Death must be reported if it occurs during the serious adverse event reporting period after the last dose of investigational product, irrespective of any intervening treatment*.

8. STATISTICAL ANALYSIS

Demographic and baseline characteristics will be summarized by treatment arm for all randomized patients using descriptive statistics.
All patients who receive at least one dose of eribulin or paclitaxel will be included in the analysis for safety. The safety analysis will report the frequency of all adverse events and the laboratory abnormalities, as well as the frequency of dose interruptions, dose reductions and treatment discontinuation for toxicity in each treatment arm. Toxicity rates will be presented using the worst NCI-CTCA grade per patient.

The efficacy endpoint of this randomized phase II neoadjuvant study in women with early stage breast cancer not overexpressing HER-2 is pathologic complete response (pCR). Patients will be randomized in a 1:1 ratio electronically into two groups by the University Of Texas MD Anderson Cancer Center's Clinical Oncology Research System (CORe).
Group 2 will receive sequential Eribulin followed by FAC/FEC-regimen chemotherapy and Group 1 will receive sequential paclitaxel followed by FAC/FEC-regimen chemotherapy (MDACC standard of care).
The primary objective is to compare the pCR rate of eribulin followed by FAC/FEC-regimen to the pCR rate of paclitaxel followed by FAC/FEC-regimen in HER-2 negative women with operable breast cancer. A binomial superiority two-sample test for population proportions will be used to compare the pCR rates of the two groups.
An interim futility analysis is planned for this two-stage trial after the endpoint has been evaluated for the first 46 surgically evaluable patients. If the test statistic at the end of the first stage is less than -0.876, the trial will stop for futility; else, the trial will continue until 152 surgically evaluable patients are accrued. A test statistic greater than 1.272 at the end of the second stage implies that the pCR rate for Group 2 is significantly better.

These calculations assume:

1. A one-sided z-test based on a pooled estimate of the variance,
2. An alpha error of 0.10,
3. 80% power,
4. A treatment arm assignment fraction of 50%, and
5. An alpha spending function based on the O'Brien-Fleming boundary.

The pCR rate of Group 1 is assumed to be 18% and the trial is powered to detect an improvement of the 15 percentage points for the pCR rate (i.e., a pCR rate of 33% for Group 2). We expect accrual to be approximately 6 patients per month. As a secondary objective, the rate of breast conservation surgery will be estimated for each group and a 95% confidence interval for the rate of breast conservation surgery for each group will be reported.

We expect accrual to be approximately 6 patients per month.
As a secondary objective, the rate of breast conservation surgery will be estimated for each group and a 95% confidence interval for the rate of breast conservation surgery for each group will be reported.

Death occurring prior to surgery is considered a treatment failure.

9. PUBLICATION OF TRIAL RESULTS

Publications resulting from this trial may be developed by the investigator who will provide Eisai an opportunity (within 60 days before submission or other public disclosure) to prospectively review any proposed publication, abstract or other type of disclosure that reports the results of the study.

9.0 STUDY CALENDAR

STUDY WEEK		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	26	28
STUDY DAY	**-14 to 0**	1	8	15	22	29	36	43	50	57	64	71	78	85	92	99	106	113	120	127	134	141	148	155	162		
Eribulin(+/- 2) [5]		X	X		X	X		X	X		X	X															
Paclitaxel(+/- 2) [5]		X	X	X	X	X	X	X	X	X	X	X	X														
FAC/FEC Regimen(+/-3) [5]														X			X			X			X				
Informed Consent	X																										
Demographics	X																										
Medical History	X																										
General Physical	X	X*			x			X			x			X			X			X			X				
Vitals Signs, Weight	X	X*			x			X			x			X			X			X			X				
Performance Status	X	X*			x			X			x			X			X			X			X				
Baseline Symptoms / Toxicities	X	X*			X			X			x			X			X			X			X				
CBC	X	X*	X	X[1]	X	X	X[1]	X	X	X[1]	X	X	X[1]	X			X			X			X				
Chemistries[2]	X	X*			x			x			X			X			X			X			X				
Pregnancy Test (Serum)	X																										
Cardiac Scan (MUGA or 2DEcho)	X													X													
EKG	X																										
Breast Ultrasound	X													X													
Core and FNA Biopsy and Correlative studies	x													X													
CTC Optional[3]	X													X													
Surgery/RCB[4]	See footnote																										
*If performed > 10 days before start the treatment																											

[1] CBC only for Paclitaxel group

[2] Chemistries: Albumin, BUN, Calcium, Creatinine, Total Bilirubin, Alk. Phosphatase, ALT (SGPT), AST (SGOT), Electrolytes (Sodium, Potassium, Chloride, CO2,) Magnesium, Glucose

[3] Will follow patients toxicities 2 weeks after surgery when follow-up scheduled with surgeon and collect third CTC at this time.

[4] Surgery/RCB performed during Weeks 26-28

5 Add allowable +/- days in the treatment schedule

11. REFERENCES

1. Cortes, J., et al., *Eribulin monotherapy versus treatment of physician's choice in patients with metastatic breast cancer (EMBRACE): a phase 3 open-label randomised study.* Lancet, 2011. **377**(9769): p. 914-23.
2. *American Cancer Society: Cancer Facts and Figures*. 2006 [05 April 2006].
3. Jemal, A., et al., *Cancer statistics, 2004.* CA Cancer J Clin, 2004. **54**(1): p. 8-29.
4. Mauri, D., N. Pavlidis, and J.P. Ioannidis, *Neoadjuvant versus adjuvant systemic treatment in breast cancer: a meta-analysis.* J Natl Cancer Inst, 2005. **97**(3): p. 188-94.
5. Singletary, S.E., M.D. McNeese, and G.N. Hortobagyi, *Feasibility of breast-conservation surgery after induction chemotherapy for locally advanced breast carcinoma.* Cancer, 1992. **69**(11): p. 2849-52.
6. Bear, H.D., *Indications for neoadjuvant chemotherapy for breast cancer.* Semin Oncol, 1998. **25**(2 Suppl 3): p. 3-12.
7. Fisher, B., et al., *Effect of preoperative chemotherapy on the outcome of women with operable breast cancer.* J Clin Oncol, 1998. **16**(8): p. 2672-85.
8. Kaufmann, M., et al., *Recommendations from an international expert panel on the use of neoadjuvant (primary) systemic treatment of operable breast cancer: an update.* J Clin Oncol, 2006. **24**(12): p. 1940-9.
9. Buzdar, A.U., *Preoperative chemotherapy treatment of breast cancer--a review.* Cancer, 2007. **110**(11): p. 2394-407.
10. Gralow, J.R., et al., *Preoperative therapy in invasive breast cancer: pathologic assessment and systemic therapy issues in operable disease.* J Clin Oncol, 2008. **26**(5): p. 814-9.
11. Gunduz, N., B. Fisher, and E.A. Saffer, *Effect of surgical removal on the growth and kinetics of residual tumor.* Cancer Res, 1979. **39**(10): p. 3861-5.
12. Fisher, B., N. Gunduz, and E.A. Saffer, *Influence of the interval between primary tumor removal and chemotherapy on kinetics and growth of metastases.* Cancer Res, 1983. **43**(4): p. 1488-92.
13. Feldman, L.D., et al., *Pathological assessment of response to induction chemotherapy in breast cancer.* Cancer Res, 1986. **46**(5): p. 2578-81.
14. Kuerer, H.M., et al., *Clinical course of breast cancer patients with complete pathologic primary tumor and axillary lymph node response to doxorubicin-based neoadjuvant chemotherapy.* J Clin Oncol, 1999. **17**(2): p. 460-9.
15. Bear, H.D., et al., *Sequential preoperative or postoperative docetaxel added to preoperative doxorubicin plus cyclophosphamide for operable breast cancer:National Surgical Adjuvant Breast and Bowel Project Protocol B-27.* J Clin Oncol, 2006. **24**(13): p. 2019-27.
16. Sataloff, D.M., et al., *Pathologic response to induction chemotherapy in locally advanced carcinoma of the breast: a determinant of outcome.* J Am Coll Surg, 1995. **180**(3): p. 297-306.
17. Symmans, W.F., et al., *Measurement of residual breast cancer burden to predict survival after neoadjuvant chemotherapy.* J Clin Oncol, 2007. **25**(28): p. 4414-22.
18. Fisher, B., et al., *Effect of preoperative chemotherapy on local-regional disease in women with operable breast cancer: findings from National Surgical Adjuvant Breast and Bowel Project B-18.* J Clin Oncol, 1997. **15**(7): p. 2483-93.

19. Wolmark, N., et al., *Preoperative chemotherapy in patients with operable breast cancer: nine-year results from National Surgical Adjuvant Breast and Bowel Project B-18.* J Natl Cancer Inst Monogr, 2001(30): p. 96-102.
20. Bear, H.D., et al., *The effect on tumor response of adding sequential preoperative docetaxel to preoperative doxorubicin and cyclophosphamide: preliminary results from National Surgical Adjuvant Breast and Bowel Project Protocol B-27.* J Clin Oncol, 2003. **21**(22): p. 4165-74.
21. van der Hage, J.A., et al., *Preoperative chemotherapy in primary operable breast cancer: results from the European Organization for Research and Treatment of Cancer trial 10902.* J Clin Oncol, 2001. **19**(22): p. 4224-37.
22. Gianni, L., et al., *Feasibility and tolerability of sequential doxorubicin/paclitaxel followed by cyclophosphamide, methotrexate, and fluorouracil and its effects on tumor response as preoperative therapy.* Clin Cancer Res, 2005. **11**(24 Pt 1): p. 8715-21.
23. Gianni, L., et al., *Phase III trial evaluating the addition of paclitaxel to doxorubicin followed by cyclophosphamide, methotrexate, and fluorouracil, as adjuvant or primary systemic therapy: European Cooperative Trial in Operable Breast Cancer.* J Clin Oncol, 2009. **27**(15): p. 2474-81.
24. Green, M.C., et al., *Weekly paclitaxel improves pathologic complete remission in operable breast cancer when compared with paclitaxel once every 3 weeks.* J Clin Oncol, 2005. **23**(25): p. 5983-92.
25. Sparano, J.A., et al., *Weekly paclitaxel in the adjuvant treatment of breast cancer.* N Engl J Med, 2008. **358**(16): p. 1663-71.
26. Seidman, A.D., et al., *Randomized phase III trial of weekly compared with every-3-weeks paclitaxel for metastatic breast cancer, with trastuzumab for all HER-2 overexpressors and random assignment to trastuzumab or not in HER-2 nonoverexpressors: final results of Cancer and Leukemia Group B protocol 9840.* J Clin Oncol, 2008. **26**(10): p. 1642-9.
27. Buzdar, A.U., et al. *Prospective randomized trial evaluating weekly paclitaxel (WP) versus docetaxel in combination with capecitabine (DC) in operable breast cancer*. 2009: J Clin Oncol.
28. von Minckwitz, G., et al., *Doxorubicin with cyclophosphamide followed by docetaxel every 21 days compared with doxorubicin and docetaxel every 14 days as preoperative treatment in operable breast cancer: the GEPARDUO study of the German Breast Group.* J Clin Oncol, 2005. **23**(12): p. 2676-85.
29. Buzdar, A.U., et al., *Prospective evaluation of paclitaxel versus combination chemotherapy with fluorouracil, doxorubicin, and cyclophosphamide as neoadjuvant therapy in patients with operable breast cancer.* J Clin Oncol, 1999. **17**(11): p. 3412-7.
30. Amat, S., et al., *Neoadjuvant docetaxel for operable breast cancer induces a high pathological response and breast-conservation rate.* Br J Cancer, 2003. **88**(9): p. 1339-45.
31. Heys, S.D., et al., *Neoadjuvant docetaxel in breast cancer: 3-year survival results from the Aberdeen trial.* Clin Breast Cancer, 2002. **3 Suppl 2**: p. S69-74.
32. Ellis, G.K., et al., *Phase III comparison of standard doxorubicin and cyclophosphamide versus weekly doxorubicin and daily oral cyclophosphamide plus granulocyte colony-stimulating factor as neoadjuvant therapy for inflammatory and locally advanced breast cancer: SWOG 0012.* J Clin Oncol, 2011. **29**(8): p. 1014-21.
33. von Minckwitz, G., et al., *In vivo chemosensitivity-adapted preoperative chemotherapy in patients with early-stage breast cancer: the GEPARTRIO pilot study.* Ann Oncol, 2005. **16**(1): p. 56-63.

34. Miller, K.D., et al., *Randomized phase II trial of the anti-angiogenic potential of doxorubicin and docetaxel; primary chemotherapy as Biomarker Discovery Laboratory.* Breast Cancer Res Treat, 2005. **89**(2): p. 187-97.
35. Earl, H., et al. *Neo-tAnGo: A neoadjuvant randomized phase III trial of epirubicin cyclophosphamide and paclitaxel with and without gemcitabine in the treatment of women with high-risk early breast cancer; first report of the primary endpoint, pathological complete response.* 2009: J Clin Onco.
36. Alvarez, R.A., et al. *The effect of different sequencing regimens of taxanes and anthracyclines in the primary systemic treatment (PST) of breast cancer (BC) patients (pts): M. D. Anderson Cancer Center retrospective analysis*. 2010: J Clin Oncol.
37. Maeda, N., et al., *Development of a DNA barcode tagging method for monitoring dynamic changes in gene expression by using an ultra high-throughput sequencer.* Biotechniques, 2008. **45**(1): p. 95-7.