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TITLE: A Phase 2 Study of Eribulin Followed by Doxorubicin and Cyclophosphamide as Preoperative Therapy for HER2-negative Inflammatory Breast Cancer

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Statistician: [redacted]

Study Coordinator: [redacted]

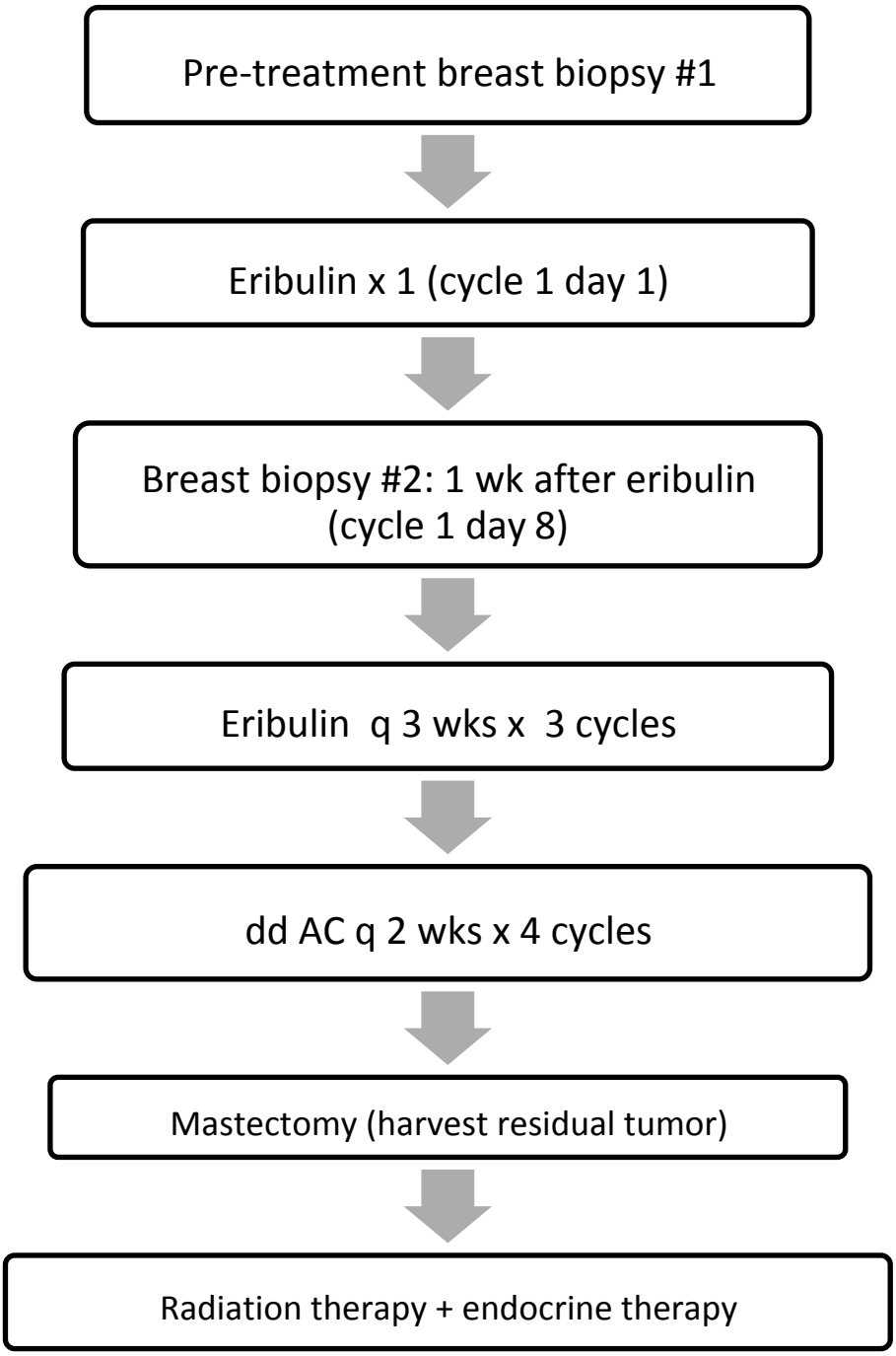
Agent(s): Eribulin, [redacted]
Doxorubicin, supplier - commercial
Cyclophosphamide, supplier - commercial

Study Exempt from IND Requirements per 21 CFR 312.2(b).

Protocol Type / Version # / Version Date: Amendment #8/Version #13/April 23, 2021

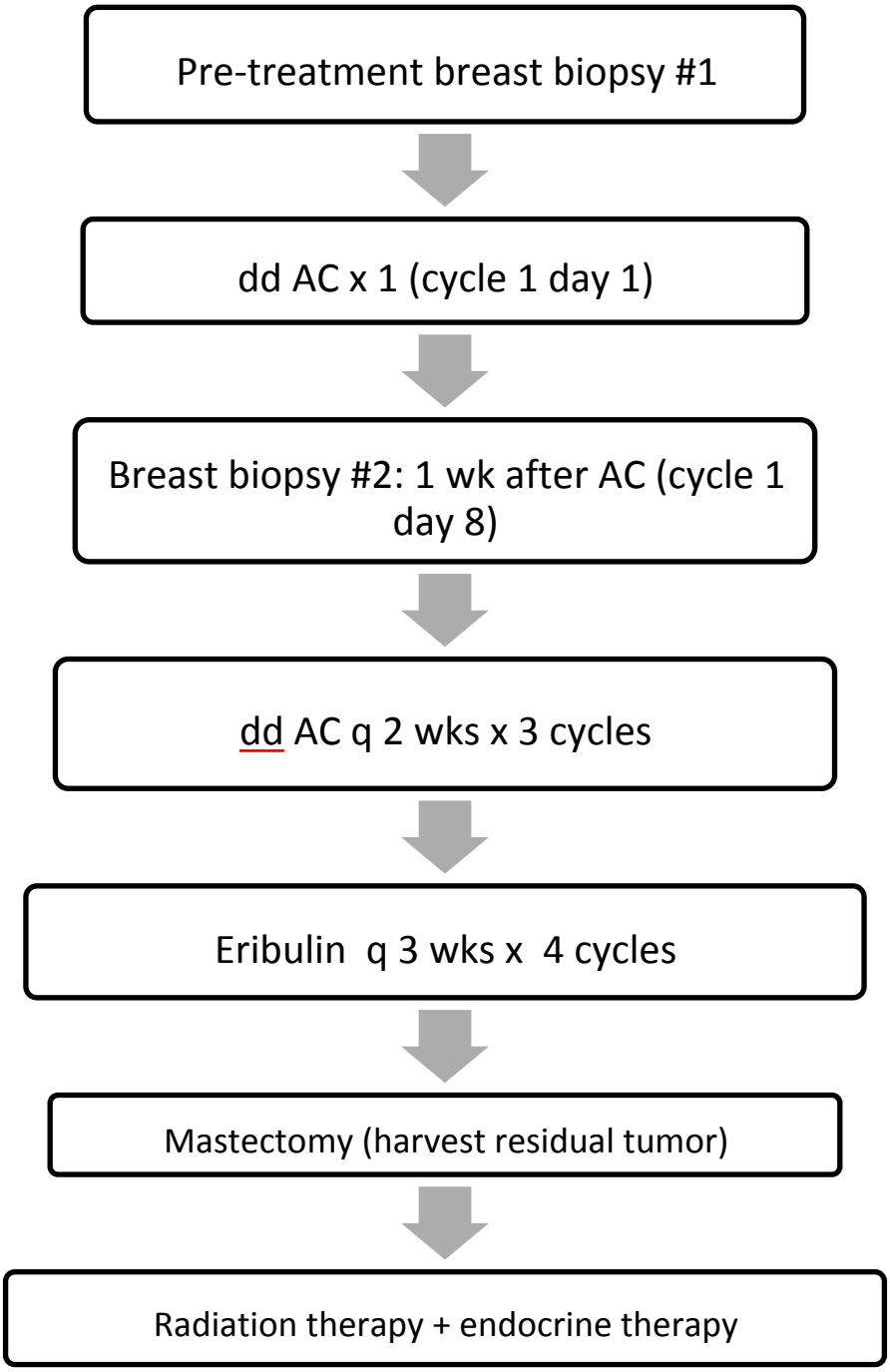
SCHEMA

Cohort A: Preoperative eribulin followed by ddAC

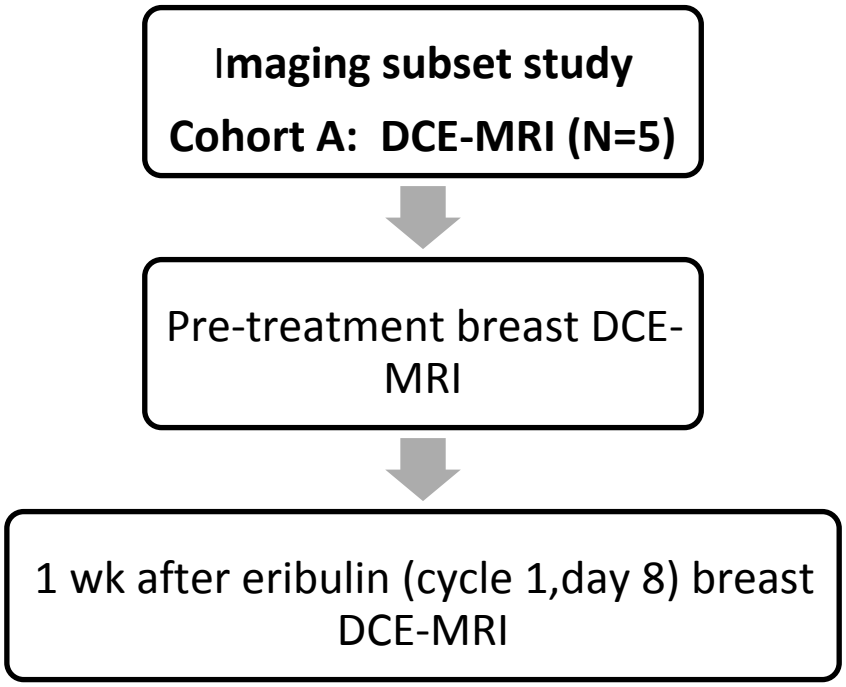


Drugs:
AC – doxorubicin + cyclophosphamide dd
– dose-dense

SCHEMA:
Cohort B: Preoperative ddAC followed by eribulin



Drugs:
AC – doxorubicin + cyclophosphamide dd
– dose-dense



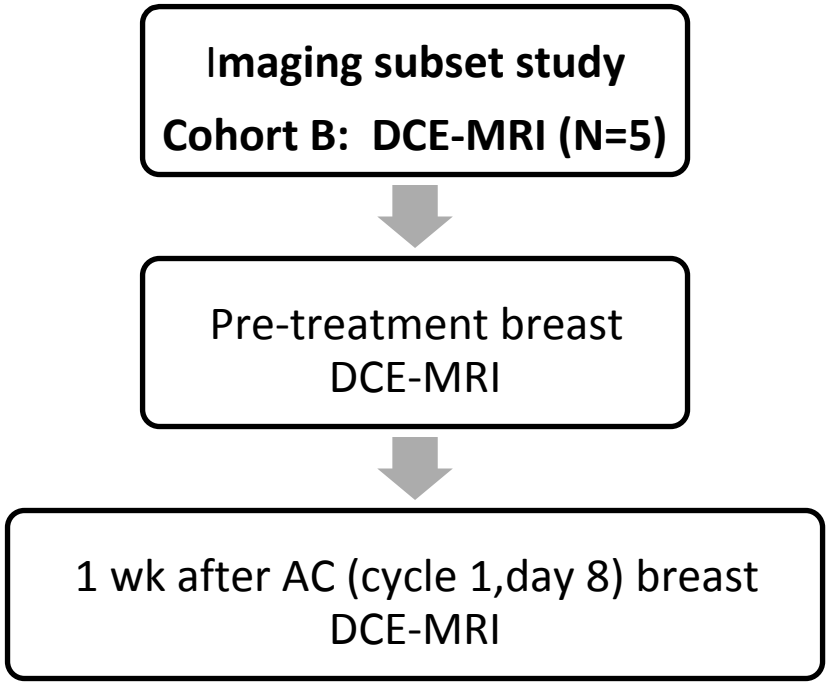


TABLE OF CONTENTS

OBJECTIVES

1.1 Study Design

Cohort A: A total of 16 patients with newly diagnosed HER2-negative inflammatory breast cancer (IBC) without visceral metastasis will receive preoperative therapy with eribulin given day 1 and day 8 every 21 days for 4 cycles followed by doxorubicin and cyclophosphamide (AC) given every 14 days (dose-dense (dd)) for 4 cycles.

- A baseline research breast biopsy is obtained prior to beginning eribulin therapy. A second research breast biopsy is obtained 8 days after the first dose of eribulin.

- A cohort of patients (5) will participate in an imaging sub-study and undergo a research DCE-MRI of the breast after the baseline research breast biopsy. A second research DCE-MRI of the breast will be performed prior to the second research biopsy of the breast.

Cohort B: A total of 16 patients with newly diagnosed HER2-negative inflammatory breast cancer (IBC) without visceral metastasis will receive preoperative therapy with doxorubicin and

cyclophosphamide (AC) given every 14 days (dose-dense (dd)) for 4 cycles followed by eribulin given day 1 and day 8 every 21 days for 4 cycles.

- A baseline research breast biopsy is obtained prior to beginning AC therapy. A second research breast biopsy is obtained 8 days after the first dose of AC.

- A cohort of patients (5) will participate in an imaging sub-study and undergo a research DCE-MRI of the breast after the baseline research breast biopsy. A second research DCE-MRI of the breast will be performed prior to the second research biopsy of the breast.

Cohort A and B:

3 to 5 weeks following the completion of preoperative study therapy, patients deemed surgically operable proceed to total mastectomy and axillary lymph node dissection, where residual cancer is obtained for correlative studies.

- Patients whose disease is not deemed surgically resectable following the completion of preoperative study therapy, may proceed to definitive radiation therapy given to the affected breast and regional lymph nodes beginning approximately 3 to 5 weeks after completion of preoperative therapy.

- Patients whose disease is rendered surgically resectable following radiation therapy may proceed to total mastectomy and axillary lymph node dissection approximately 4 to 8 weeks following the completion of radiation therapy. Residual cancer will be obtained for correlative studies.

- Definitive radiation is given to the post-surgical chest wall and regional lymph nodes beginning approximately 3 to 6 weeks after surgery.

- Endocrine therapy with tamoxifen or an aromatase inhibitor, with or without ovarian suppression, will be given for 5-10 years if estrogen receptor (ER) and / or progesterone receptor (PR) positive. Endocrine therapy will begin either during or after completing radiation therapy as per standard of care practices.

- After completion of protocol therapy, participants in both cohorts will be followed every 3 months for 1 year, then every 6 months for 4 years, then annually until death.

1.2 Primary Objectives for both Cohort A and Cohort B:

- To determine pathologic complete response (pCR) rate after preoperative therapy for HER2-negative inflammatory breast cancer.

1.3 Secondary Objectives for both Cohort A and Cohort B:

- To determine the efficacy of preoperative therapy for HER2-negative IBC defined as disease-free survival (DFS), time to treatment failure (TTF), and overall survival (OS).

- To assess the residual cancer burden (RCB) after preoperative therapy in HER2-negative inflammatory breast cancer.

1.4 Correlative Objectives for both Cohort A and Cohort B:

- To correlate levels of expression of a panel of 20 pre-selected genes encoding for proteins involved with angiogenesis or epithelial-to-mesynchaml transition (EMT) with disease outcome (pCR, RCB, DFS, OS).

- To correlate changes in imaging (K^{trans} v_e, v_p, and initial area under the curve (iAUC)) with genomic changes determined on core biopsies of the breast sampled at the same time points.

2. BACKGROUND

2.1 Inflammatory Breast Cancer

Inflammatory breast cancer (IBC) accounts for 2-5% of all invasive breast cancer. IBC is classified as a "cliniopathologic" diagnosis, whereby documentation of invasive breast carcinoma is established in the setting of unique clinical characteristics including a rapid onset of breast enlargement, pain, diffuse erythema and edema (peau d'orange) usually occurring within 3-6 months. The breast cancer often presents without a palpable mass, and dermal lymphatic involvement with cancer is demonstrated in approximately 75% of the cases.[1] It is the effect of dermal lymphatic involvement, not infiltration of inflammatory cells that result in the clinical changes observed in IBC. The median age at presentation is less than that seen in non-IBC, and there is a greater incidence of IBC among African American women. [2, 3]

The intrinsic biology of IBC is such that advanced disease is present at the time of diagnosis. Approximately 55-85% of patients present with metastasis to the axillary and / or supraclavicular lymph nodes, and distant metastatic disease is present at diagnosis in approximately 20-40% of women. [4] Even in the absence of metastatic disease at presentation, the likelihood of developing distant metastasis is extremely high, supporting a role for chemotherapy as the mainstay of treatment. Historically, surgery and radiation therapy alone resulted in a 54% relapse rate within 18 months, translating into a median survival of 1.2 years. [5] The addition of chemotherapy to the initial treatment of IBC, i.e. trimodality therapy with neoadjuvant chemotherapy, followed by mastectomy and radiation therapy, improved the median overall survival to 3.8 years, translating into an approximately 50% 5-year overall survival. [1]

The extent of locoregional disease precludes mastectomy as primary treatment for IBC, therefore preoperative chemotherapy has become the standard of care; however, the optimal chemotherapy regimen has yet to be determined. Anthracycline-containing regimens employed preoperatively have resulted in 40-45% 5-year overall survival, whereas the addition of taxanes to these regimens improved the pathologic complete response rate and overall survival. [6-8] The current chemotherapy regimens utilized as pre-operative treatment for IBC most commonly include anthracyclines and taxanes, yet the poor overall survival rates still necessitate ongoing investigation into improved pre-operative regimens with the goal of improving disease outcome.

2.2 IND Agent

Eribulin

Eribulin mesylate is a synthetic derivative of the natural product halichondrin B, a large polyether macrolide isolated from a marine sponge (*Halichondria okadai*). Halichondrin B exhibits anti-cancer activity through a microtubule-destabilizing anti-mitotic mechanism of action.[9-11] Eribulin exerts its effects by binding to the plus end of microtubules, leading to tubulin sequestration into nonproductive aggregates, preventing tubulin polymerization and microtubule dynamics. Suppression of microtubule growth interferes with normal mitotic spindle formation, and blocks the prometaphase portion of mitosis,[12] leading to irreversible cell cycle blockade at G2/M, disruption of mitotic spindles, and cell death via apoptosis after prolonged mitotic blockage.[13] *In vitro* studies demonstrate that analogues of halichondrin B inhibit cell growth at nanomolar concentrations in a wide variety of cancer cell types, including breast, ovary, colon and melanoma.[14]

The dosing regimen for eribulin selected based on results of Phase 1 studies is 1.4 mg/m2 administered i.v. on Days 1 and 8 of a 3-week cycle. In an early Phase 2 trial, E7389-A001-201, the response rate (RR) in patients with breast cancer previously treated with anthracycline and taxanes was 11.5%.[15] Based on these results, the effectiveness and safety of eribulin were further explored in Study E7389-A001-211, which included patients with locally advanced or metastatic breast cancer who had received between two to five prior chemotherapy regimens, including an anthracycline, taxane, and capecitabine, and who had progression on or within 6 months of their last chemotherapy regimen. The response rate for eribulin was 9.3% in these heavily pretreated subjects.[16]

In a Phase 3 trial, E7389-G000-305 ("EMBRACE"), eribulin (1.4 mg/m2 on Days 1 and 8 of a 3week cycle) was compared with treatment of the physician's choice (TPC; any cytotoxic or hormonal agent) in subjects heavily pre-treated for locally advanced or metastatic breast cancer.[17] In the analysis of the primary endpoint of overall survival, patients receiving eribulin had a longer median overall survival of 13.1 months compared with 10.7 months for TPC (P = 0.04, stratified log-rank test; hazard ratio = 0.81). Objective RR was 12% for eribulin mesylate and 5% for TPC (P = 0.005). Grade 3 or 4 eribulin-related adverse events included

asthenia/fatigue (7.6%), neutropenia (44%), and peripheral neuropathy (8.4%). Twelve percent of eribulin-treated subjects and 7% of TPC-treated subjects experienced treatment related serious AEs. Based on the results of this Phase 3 trial, eribulin was approved by the FDA in November 2010 for metastatic breast cancer pre-treated with at least 2 prior regimens including anthracycline and taxane.

The tolerability of eribulin and its ease of use suggest possible treatment advantages in comparison to other available agents for advanced disease (i.e. taxanes). Preclinical and clinical data suggest that eribulin mesylate may exhibit less neurotoxicity, myalgia/arthralgia, and hypersensitivity than other chemotherapeutic agents. The most common AEs with single-agent eribulin mesylate are asthenia/fatigue and neutropenia. In addition, eribulin requires minimal preparation, can be administered quickly, and does not require premedication to prevent hypersensitivity.

Dosing justification

The starting dosage of eribulin mesylate in this study, 1.4 mg/m2 administered as an i.v. infusion over approximately 2 to 5 minutes on Days 1 and 8 of a 3-week cycle, is a modification based on results of four phase 1 and four Phase 2 clinical studies of eribulin monotherapy. Four Phase 1 dose-finding studies were conducted to evaluate escalating doses of eribulin mesylate and determine the maximum tolerated dose (MTD) of eribulin mesylate as: (1) a 1-hour i.v. infusion on Days 1, 8 and 15 of a 28-day cycle (E7389-A001-101); (2) a 1-hour i.v. infusion on Day 1 of a 21-day cycle (E7389-A001-102); (3) an i.v. bolus on Days 1, 8 and 15 of a 28-day cycle (National Cancer Institute [NCI] Study 5730); and (4) a bolus infusion on Days 1 and 8 of a 21day cycle (E7389-J081-105). The MTD was determined to be 1.4 mg/m2 for the bolus injection and 1.0 to 2.0 mg/m2 for the infusion.

Eribulin mesylate 1.4 mg/m2 i.v. bolus was tested as the starting dose in four Phase 2 studies. In the first two Phase 2 studies, subjects initially received eribulin mesylate on Days 1, 8 and 15 in 28-day cycles. Because the majority of subjects receiving the 28-day dosing schedule experienced dose delays, reductions, or omissions due to neutropenia on Day 15 of the cycle, an additional cohort of subjects who received eribulin mesylate 1.4mg/m2 on Days 1 and 8 in 21day cycles was added. This schedule appeared to be better tolerated than the 28-day schedule and demonstrated antitumor activity in all four Phase 2 studies. Thus, the optimal schedule of eribulin mesylate monotherapy based on the four studies appears to be 1.4 mg/m2 administered on Days 1 and 8 in 21-day (3-week) cycles.

2.3 Other Agent(s)

Doxorubicin and cyclophosphamide (AC) – as per institutional standards

Several studies confirmed the importance of anthracycline-based neoadjuvant (NAC) for IBC.[6, 18, 19] Bauer et al. compared the outcomes of 2 cohorts of patients with IBC: 28 patients treated between 1973 and 1988 received CMF with or without vincristine and prednisone (VP) and 10

patients treated after 1988 received FAC. [18] The median OS was significantly improved when anthracyclines were added, compared with CMF/VP (30 months vs. 18 months, respectively, p = .02), as was the 3-year OS (41% vs. 14%, respectively) and 3-year DFS (46% vs. 0%, respectively). The introduction of taxanes into the NAC armamentarium also resulted in improved outcomes. A retrospective review of 6 sequential clinical trials involving 240 patients with IBC treated at MD Anderson Cancer Center from 1973 and 2000, compared disease outcomes among patients treated with anthracycline-based NAC with or without paclitaxel (T).[8] The median PFS was improved with the addition of T (33 weeks with T vs. 26 weeks, p = .18), as was the median OS (52 weeks with T vs. 41 weeks, p = .11). Statistical significance was demonstrated in the ER negative sub-population. Horvath et al. also retrospectively compared their experience of anthracycline-based NAC versus docetaxel (D) with epirubicin (E) and found an improved 3-year OS with the taxane-based NAC; however, statistical significance was not achieved (75% with DE vs. 61%).[20] Currently, the standard NAC for IBC includes an anthracycline-and taxane-based regimen, although the optimal combination or sequence of these agents has not been established.

2.4 Rationale

Eribulin (E7389, Halaven) is a synthetic analog of Halichondrin B that suppresses microtubule growth, without effecting polymerization.[21] Because of its unique mechanism of action, i.e., sequestration of tubulin into nonfunctional aggregates, eribulin has been found to be an effective agent against taxane resistant cancer. Two separate phase II studies of eribulin in the treatment breast cancer patients previously exposed to taxanes, anthracyclines, and capecitabine demonstrated significant response rates, prompting the EMBRACE trial which showed a survival advantage in highly pre-treated patients.[19, 22, 23] These data strongly support the investigation of eribulin in IBC; a virulent and often chemo-resistant subtype of breast cancer. We hypothesize that the use of eribulin will result in a significant improvement in pCR compared with the historical outcome of 25%.[8, 19] The improved pCR rate should translate into an improved disease-free survival and overall survival.

Only 1/16 subjects enrolled in Cohort A achieved a pCR, therefore we did not proceed to the second stage of study enrollment. This prompted an updated assessment of the expected pCR from preoperative therapy for hormone receptor positive / HER2 negative IBC. More recent studies of preoperative therapy and its effect on subtypes of inflammatory breast cancer, has shown that the pCR rate of ER and / or PR positive, HER2 negative IBC is significantly less than 10%.[24] Our analysis of the DFCI IBC Registry (DF/HCC 11-035) demonstrated a pCR rate among the hormone receptor positive, HER2 negative subgroup to be 2%. This data supported the pCR rate seen in Cohort A, therefore we sought to expand the protocol to explore a drug sequencing question and link it with an exploration of impact on the correlative studies. The original protocol has been modified to include a Cohort B which changes the sequencing of the preoperative chemotherapy to ddAC followed by eribulin. The Correlative Studies will remain the same, which will allow a comparison of gene expression following eribulin administration versus AC chemotherapy exposure. An additional 16 patients will be enrolled in Cohort B.

2.5 Correlative Studies Background

Eribulin mesylate is a synthetic macrocytic ketone analogue of the marine sponge natural product halichondrin B, and an inhibitor of microtubule dynamics. By inhibiting microtubular mechanics, eribulin exerts its primary effect by preventing cancer cells' progression through the cell cycle, leading to apoptosis. Importantly, eribulin has shown evidence of clinical efficacy against tumors that have progressed on other microtubule-targeted cytotoxics such as taxanes. Recently, preclinical work has revealed two other potential mechanisms of action for eribulin.

Eribulin induces a mesenchymal-to-epithelial transition

Recently published data suggests that eribulin can induce a shift in the phenotype of triple-negative breast cancer (TNBC) cells from a mesenchymal state to a more epithelial state. [25] This "mesenchymal-to-epithelial transition" (MET) represents a shift in cellular phenotype from a motile, invasive, stem-like state to a less invasive epithelial one [26, 27]. In both mice and humans, evidence suggests that induction of MET might be associated with a reduction in tumor cell extravasation into the circulation and the formation of distant metastases [26, 28].

In preclinical studies, TNBC cells treated with eribulin *in vitro* show a downregulation (at both the mRNA and protein levels) of mesenchymal markers including N-Cadherin, vimentin, Twist, Snail, ZEB1, and ZEB2 and a concomitant increase in the expression of classical epithelial markers including E-Cadherin and keratin-18. Consistent with this, eribulin pre-treated cells showed a reduction in migratory and invasive capacities, and a relative ineptitude for colonizing distant organs and forming micrometastatic lesions [25]. Although speculative, this might relate to an eribulin-induced attenuation of Smad2/3 phosphorylation by TGF-beta [25]. Importantly, none of the described changes were observed when the same cells were treated with a cytotoxic with an alternate mechanism of action, 5-fluorouracil.

2.5.2 Eribulin normalizes the vasculature of primary mammary tumors

Preclinical work has also revealed that eribulin might have direct or indirect effects on mammary tumor vessels [29]. In two murine TNBC xenograft models, eribulin therapy was associated with an improvement of blood perfusion in the central region of tumors, an increase in vessel density, a reduction in vessel diameter, and a reduction in hypoxia. Collectively, these changes are consistent with "vascular normalization" – a reversion of tumor vessels from an immature, abnormal phenotype to one more reminiscent of normal vessels [30]. Importantly this normalization was associated with an increased effect of concomitantly administered cytotoxics, and was not seen after treating tumors with capecitabine, a cytotoxic with a different mechanism of action.

2.5.3 Proposed translational experiments

Collectively, the above data provide intriguing insights into alternate and potentially unique mechanisms of action for eribulin. In the current study - *A Phase 2 Study of Eribulin Followed by Doxorubicin and Cyclophosphamide as Preoperative Therapy for HER2-negative Inflammatory*

Breast Cancer – we propose a series of correlative experiments seeking evidence of the abovementioned changes in response to eribulin treatment of human inflammatory breast cancer (IBC). An imaging sub-study will also be performed to evaluate the effects of eribulin on tumor vasculature. IBC represents an ideal model as it demonstrates both a high metastatic proclivity and robust angiogenesis.

Although the preclinical data supports a specific effect of eribulin on gene expression and vascular normalization, these changes may be due to chemotherapy in general, therefore the same correlative analysis described above will be performed on biopsy samples and breast imaging after exposure to AC preoperative therapy.

2.5.4 Additional Biomarker Analysis

The study also requires mandatory blood samples for biomarker research, which may include, among other analyses, the assessment of circulating tumor DNA. There is increasing evidence that circulating DNA can be obtained from the blood specimens of cancer patients, which represents the DNA and mutational status of tumor cells[37-42]. The serial sampling gives the opportunity to evaluate changes in circulating biomarker levels over time that may allow further understanding of potential resistance mechanisms or of (early) indicators of recurrence. Additional candidate markers of response to treatments that emerge from other clinical or nonclinical studies may also be assessed in this study and can be assessed with different types of technologies.

3. PARTICIPANT SELECTION (FOR BOTH COHORTS A AND B)

3.1 Eligibility Criteria

Participants must have histologically confirmed invasive breast cancer. All histologic subtypes are eligible.

Participants must NOT have HER2 positive status based on ASCO/CAP guidelines defined as:

- IHC 3+ based on circumferential membrane staining that is complete, intense

-AND/OR –

- FISH positive based on one of the three following criteria: ○ Single-probe average HER2 copy number ≥ 6.0 signals/cell; **OR**
  - Dual-probe HER2/CEP17 ratio <2.0 with an average HER2 copy number ≥ 6.0 signals/cell; **OR**
  - Dual-probe HER2/CEP17 ratio ≥2.0

Age ≥ 18 years. Because no dosing or adverse event data are currently available on the use of eribulin in participants <18 years of age, children are excluded from this study

ECOG performance status ≤1 (Karnofsky ≥70%, see Appendix A)

Participants must have normal organ and marrow function as defined below:

- leukocytes	≥3,000/mcL
- absolute neutrophil count	≥1,500/mcL
- platelets total	≥100,000/mcL
- bilirubin	within normal institutional limits
- AST(SGOT)/ALT(SGPT)	≤2.5 × institutional upper limit of normal
- creatinine OR	≤1.5 x institutional upper limit of normal
- creatinine clearance	≥60 mL/min/1.73 m^2 for participants with creatinine levels above institutional normal.

Patients must have the clinical diagnosis of inflammatory breast cancer.

Patients must be without evidence of visceral or bone involvement with metastatic cancer on physical exam or any diagnostic study. Extensive nodal involvement (distant or regional) is allowed.

LVEF ≥ 50% calculated by echocardiogram (ECHO)

Patients may have bilateral breast cancer so long as one breast meets criteria for inflammatory breast cancer, and the breast with inflammatory breast cancer has never received prior therapy.

The effects of eribulin on the developing human fetus are unknown. For this reason and because other therapeutic agents used in this trial are known to be teratogenic, women of childbearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately. Men treated or enrolled on this protocol must also agree to use adequate contraception prior to the study, for the duration of study participation, and 4 months after completion of chemotherapy administration.

Ability to understand and the willingness to sign a written informed consent document.

3.2 Exclusion Criteria

Participants who are receiving any other investigational agents.

History of allergic reactions attributed to compounds of similar chemical or biologic composition to eribulin or other agents used in study.

Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

Pregnant women are excluded from this study because eribulin is an agent with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with eribulin, breastfeeding should be discontinued if the mother is treated with eribulin. These potential risks may also apply to other agents used in this study.

HIV-positive participants on combination antiretroviral therapy are ineligible because of the potential for pharmacokinetic interactions with eribulin. In addition, these participants are at increased risk of lethal infections when treated with marrow-suppressive therapy. Appropriate studies will be undertaken in participants receiving combination antiretroviral therapy when indicated.

A baseline corrected QT interval of > 500 ms. Patients with left bundle branch block that is deemed not clinically significant may be enrolled with corrected QT > 500.

Individuals with a history of a different malignancy are ineligible except for the following circumstances. Individuals with a history of other malignancies are eligible if they have been disease-free for at least 3 years and are deemed by the investigator to be at low risk for recurrence of that malignancy. Individuals with the following cancers are eligible if diagnosed and treated within the past 3 years: cervical cancer in situ, and basal cell or squamous cell carcinoma of the skin.

Patients may not have received eribulin, paclitaxel, doxorubicin, or cyclophosphamide as antineoplastic therapy.

3.3 Inclusion of Women and Minorities

Both men and women of all races and ethnic groups are eligible for this trial. Because breast cancer predominantly affects females, it is anticipated that male enrollment will be < 5% of the overall study population.

4. REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC and DF/PCC Institutions

Institutions will register eligible participants in the Clinical Trials Management System (CTMS) OnCore. Registrations must occur prior to the initiation of protocol therapy. Any participant not registered to the protocol before protocol therapy begins will be considered ineligible and registration will be denied.

An investigator will confirm eligibility criteria and a member of the study team will complete the protocol-specific eligibility checklist.

Following registration, participants may begin protocol therapy. Issues that would cause treatment delays should be discussed with the Overall Principal Investigator (PI).

4.2 Registration Process for DF/HCC

DF/HCC Standard Operating Procedure for Human Subject Research Titled *Subject Protocol Registration* (SOP #: REGIST-101) must be followed.

5. TREATMENT PLAN

5.1 Treatment Regimen

Cohort A: Eribulin will be administered on day 1 and day 8, every 3 weeks, with 21 consecutive days defined as a treatment cycle. Eribulin will be administered for a total of 4 cycles. Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 7. Appropriate dose modifications are described in Section 6.

Following the completion of eribulin, doxorubicin and cyclophosphamide (AC) will be administered on day 1, every 2 weeks, with 14 consecutive days defined as a treatment cycle. If AC is delayed due to toxicity and an investigator prefers drug administration every 3 weeks in order to maintain full dose, AC may be given on a 21 day cycle with approval of the Principal Investigator. AC will be administered for a total of 4 cycles. Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 7. Appropriate dose modifications are described in Section 6. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the participant's malignancy.

Cohort B: Doxorubicin and cyclophosphamide (AC) will be administered on day 1, every 2 weeks, with 14 consecutive days defined as a treatment cycle. If AC is delayed due to toxicity

and an investigator prefers drug administration every 3 weeks in order to maintain full dose, AC may be given on a 21 day cycle with approval of the Principal Investigator. AC will be administered for a total of 4 cycles. Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 7. Appropriate dose modifications are described in Section 6. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the participant's malignancy.

Following the completion of AC, eribulin will be administered on day 1 and day 8, every 3 weeks, with 21 consecutive days defined as a treatment cycle. Eribulin will be administered for a total of 4 cycles. Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 7. Appropriate dose modifications are described in Section 6.

Treatment					
Agent	**Premedications; Precautions**	**Dose**	**Route**	**Schedule**	**Cycle Length**
Eribulin	No routine premedication required	1.4 mg/m^2 – may be administered diluted in 100 ml normal saline	IV over 2-5 minutes.	Day 1 and day 8, every 21 days x 4 cycles	21 days (3weeks)
Doxorubicin	No routine premedication required	60 mg/m2, IVP	IVP over 3-5 minutes; given prior to cyclophosphamide	Day 1, every 14 days x 4 cycles	14 days (2weeks)
Cyclophosphamide	No routine premedication required	600 mg/m2 diluted in 250500 ml normal saline	IV infusion administered per institutional guidelines; given after doxorubicin	Day 1, every 14 days x 4 cycles	

- Minor schedule changes owing to observed holidays, inclement weather, etc. are permitted.
- Patients may interrupt therapy for protocol-directed reasons (i.e. toxicity) or for personal preferences (holidays, vacations, etc). Treatment should resume according to protocol guidelines.
- [redacted] will provide eribulin mesylate to DFCI Pharmacy for use in the study for dispensing free of cost to participants. Patients and/or their insurance companies will be billed for the cost of doxorubicin and cyclophosphamide and their administration, as it is considered standard of care for inflammatory breast cancer.

5.2 Pre-Treatment Criteria (for both Cohort A and B)

Cycle 1-8, Day 1

- Physical examination will be performed.

At every visit, vital signs and laboratory tests will be performed. Pretreatment criteria is required at day 1 of every cycle (including C1D1) and on day 8 when eribulin is being administered and will include:

- Absolute neutrophil count > 1,000/mcL.
- Platelets > 75,000/mcL.
- Total bilirubin ≤1.5 X institutional upper limit of normal.

  Alkaline phosphatase (ALP) ≤3 x institutional upper limit of normal.
- AST(SGOT)/ALT(SGPT) ≤ 3 X institutional ULN.
- Creatinine ≤ 2.0 x ULN or creatinine clearance ≥50 mL/min.
- Non-hematologic toxicity CTCAE < Grade 3 or 4.

5.3 Agent Administration

Eribulin

- Eribulin at a starting dose of 1.4 mg/m^2 will be administered on an outpatient basis via IV infusion per institutional guidelines on Days 1 and 8 of each 21 day cycle, for a total of 4 cycles.
  - Cohort B: First dose of eribulin will be administered 2 weeks after the last dose of AC is given (cycle 4, day 1).
- Eribulin dosing will be administered per institutional guidelines.

Other agents

Doxorubicin

- Doxorubicin and cyclophosphamide are administered concurrently (AC).

-
    - Cohort A: AC will be administered beginning 2 weeks after the last dose of eribulin is given (cycle 4, day 8).

- Doxorubicin 60 mg/m2 will be administered on an outpatient basis via IV. per institutional guidelines on Day 1 of each 14 day cycle, for a total of 4 cycles.

  Doxorubicin is administered immediately prior to the administration of cyclophosphamide.

Cyclophosphamide

- Doxorubicin and cyclophosphamide are administered concurrently (AC).
    - Cohort A: AC will be administered beginning 2 weeks after the last dose of eribulin is given (cycle 4, day 8).

- Cyclophosphamide 600 mg/m2 will be administered on an outpatient basis via IV infusion per institutional guidelines on Day 1 of each 14 day cycle, for a total of 4 cycles.

- Cyclophosphamide is administered immediately following the administration of doxorubicin.

Other Modality(ies) or Procedures

Surgery

- Primary breast surgery should be performed within 3 - 5 weeks after the last dose of chemotherapy (doxorubicin/cyclophosphamide for Cohort A, eribulin for Cohort B). Surgery must be performed at one of the participating institutions. Prior to surgery, patients should be assessed for clinical response to preoperative treatment by MRI of the breasts. Patients should undergo surgery with a total mastectomy; removal of the breast and level 1 and 2 axillary lymph node dissection. Pathological specimens will be analyzed for tumor extent and grade, ER and PR status, HER2 expression, and other markers of tumor biology. Given the high risk of local regional disease recurrence with IBC, reconstruction surgery is to be delayed for 6 – 12 months following the completion of radiation therapy.

- Patients whose disease is not surgically resectable following the completion of preoperative study therapy, may proceed to definitive radiation given to the affected

- breast and regional lymph nodes beginning approximately 3 to 4 weeks after the last dose of preoperative chemotherapy. Patients whose disease is rendered surgically resectable following radiation therapy may proceed to total mastectomy and axillary lymph node dissection (as described above) approximately 4 to 8 weeks following the completion of radiation therapy.

Radiation Therapy

- Radiation therapy should be initiated within 3-6 weeks following surgery. Radiation should be delivered to the chest wall and regional lymph nodes. Treatment will be administered per institutional guidelines.Radiation can be administered at a local facility.
  Patients whose disease is not surgically resectable following the completion of preoperative study therapy may proceed to definitive radiation given to the affected breast and regional lymph nodes beginning approximately 3 to 4 weeks after completion of preoperative chemotherapy.

Endocrine Therapy

- Appropriate postoperative adjuvant endocrine therapy should be administered in patients with ER and/or PR positive disease as defined by institutional guidelines.

- Either tamoxifen or an aromatase inhibitor, with or without ovarian suppression, should be administered as determined by the patient's treating physician.

- Ovarian suppression should be offered to premenopausal patients who continue to have intact ovarian function following the completion of preoperative chemotherapy. Either tamoxifen or an aromatase inhibitor should be administered concurrently.

- This should be initiated 2-4 weeks following the completion of surgery and may be given concurrent with radiation therapy as per investigator discretion. Endocrine therapy should continue per institutional guidelines.

5.4 General Concomitant Medication and Supportive Care Guidelines

The weak inhibitory effect on cytochrome P450 enzymes exhibited by eribulin in vitro suggests a low risk of eribulin interaction with the pharmacokinetics of other drugs coadministered in usual clinic practice.

All diagnostic, therapeutic, or surgical procedures relating to malignancy should be recorded in the electronic case report form (eCRF), including the date, indication, description of the procedure(s), and any clinical findings. All prior treatment or medication administered during the 30 days preceding the first dose of study treatment and any concomitant therapy administered to

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the subject throughout the study until 30 days after the final dose of study treatment must be recorded on the Prior and Concomitant Therapy page of the eCRF. The generic name of the drug (or trade name for combination drugs) must be specified along with the duration of treatment and indication for use. If concomitant medication/therapy is administered for an AE, investigators will record that AE on the AE page of the eCRF.

Any medication that is considered necessary for the subject's welfare and that is not expected to interfere with the evaluation of study treatment may be given at the discretion of the investigator. Ancillary treatments will be given as medically indicated.

Any changes in documented, permitted concomitant treatment already being taken at the start of the clinical study must be recorded on the eCRF, noting the type of medication, duration of use, and indication.

- **Antiemetics**

Patients may be given antiemetics at the discretion of the treating physician.

- **Anticoagulants**

Anticoagulation with heparin, heparin derivatives, and/or warfarin may be given at the discretion of the treating physician. Patients receiving warfarin with therapeutic INR should have PT-INR checks according to standard institutional practice to maintain the target INR as determined by the treating investigator.

- **Growth Factors**

Patients may not receive erythropoietin while on study. The use of G-CSF should generally be administered to maintain neutrophil counts during eribulin administration as per ASCO guidelines. G-CSF should be routinely administered to maintain a 14 day cycle for doxorubicin/cyclophosphamide per institutional guidelines. The use of platelet growth factors are specifically prohibited on this protocol. Please consult the Principal Investigator with questions. Investigational growth factors are not permitted on this study.

5.5 Criteria for Taking a Participant Off Protocol Therapy

Duration of therapy will depend on individual response, evidence of disease progression and tolerance. In the absence of treatment delays due to adverse event(s), treatment may continue for a total of 8 cycles of preoperative chemotherapy (Cohort A: eribulin for 4 cycles followed by doxorubicin/cyclophosphamide (AC) for 4 cycles; Cohort B: doxorubicin/cyclophosphamide (AC) for 4 cycles followed by eribulin for 4 cycles) or until one of the following criteria applies:

- Disease progression
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Participant demonstrates an inability or unwillingness to comply with the medication regimen and/or documentation requirements
- Participant decides to withdraw from the protocol therapy
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the judgment of the treating investigator

Participants will be removed from the protocol therapy when any of these criteria apply. The reason for removal from protocol therapy, and the date the participant was removed, must be documented in the case report form (CRF). Alternative care options will be discussed with the participant.

A ODQ Treatment Ended/Off Study Form will be filled out when a participant is removed from protocol therapy. This form can be found on the ODQ website or obtained from the ODQ registration staff.

In the event of unusual or life-threatening complications, treating investigators must immediately notify the Overall PI, Filipa Lynce M.D. at 617-632-3800.

Preoperative chemotherapy

Patients who discontinue chemotherapy due to toxicity or develop disease progression should not systematically be withdrawn from all study treatments. The following recommendations are given:

Cohort A:

- Eribulin: patients who received at least 2 cycles of eribulin can proceed to doxorubicin/cyclophosphamide. Patient should then complete the treatment as per protocol.

- Doxorubicin / cyclophosphamide: in the case of discontinuation of doxorubicin/cyclophosphamide, patients should be assessed for eligibility for mastectomy, and if they are deemed surgical candidates, then they should proceed to mastectomy and continue on treatment as per protocol.

Cohort B:

- Doxorubicin / cyclophosphamide (AC): patients who received at least 2 cycles of AC can proceed to eribulin. Patients should then complete the treatment as per protocol.

- Eribulin: in the case of discontinuation of eribulin, patients should be assessed for eligibility for mastectomy, and if they are deemed surgical candidates, then they should proceed to mastectomy and continue on treatment as per protocol.

5.6 Duration of Follow-Up

After removal from protocol therapy, participants will be followed every 3 months for 1 year, then every 6 months for 4 years, then annually until death. Participants removed from protocol therapy for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event.

Patients who are no longer being followed at the treating institution will be contacted either directly or their current treating physician will be contacted to determine disease status until death.

5.7 Criteria for Taking a Participant Off Study

Participants will be removed from study when any of the following criteria apply:

- Lost to follow-up
- Withdrawal of consent for data submission
- Death

The reason for taking a participant off study, and the date the participant was removed, must be documented in the case report form (CRF).

A ODQ Treatment Ended/Off Study Form will be filled out when a participant comes off study. This form can be found on the ODQ website or obtained from the ODQ registration staff.

6. DOSING DELAYS/DOSE MODIFICATIONS FOR COHORT A AND B

Dose delays and modifications will be made as indicated in the following table(s). If any of the individual study drugs must be delayed for a day or more, all agents should be delayed for the same timeframe. Dose adjustments are to be made according to the highest grade toxicity or hematologic toxicity, whichever is more likely to result in a dose reduction. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for dose delays and dose modifications. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

If possible, symptoms should be managed symptomatically. In the case of toxicity, appropriate medical treatment should be used (including anti-emetics, anti-diarrheals, etc.).

All adverse events experienced by participants will be collected from the time of the first dose of study treatment, through the study and until the final study visit. Participants continuing to experience toxicity at the off study visit may be contacted for additional assessments until the toxicity has resolved or is deemed irreversible.

6.1 Eribulin

The principle is to attempt to administer full doses of therapy on schedule. No more than 2 dosereductions of eribulin mesylate chemotherapy should be made for toxicity. **Growth factor support may be used instead of a dose reduction at the discretion of the investigator.** If more than 2 dose-reductions would be required, eribulin should be discontinued.

- If eribulin is held for > 2 weeks for toxicity, eribulin should be discontinued.

- If eribulin is discontinued, the patient should proceed to doxorubicin/cyclophosphamide (AC) and proceed as outlined in the protocol (Cohort A) or proceed to surgery as outlined in the protocol (Cohort B).
- All dose modifications for eribulin are based on the dose level changes outlined below (Table 1).
- Instructions for management of toxicities related to eribulin are listed in Table 2.

If the dose of eribulin cannot be administered as planned due to treatment-related toxicity, the dose should be delayed according to the following instructions:

- **Day 1 of each cycle:** If eribulin cannot be administered on day 1, the dose should be delayed until recovery to above mentioned values (criteria for eribulin administration). The day 1 dose will be rescheduled for when the criteria for eribulin administration are being met. The dose of eribulin may have to be reduced following a dose delay in accordance to the instructions for dose reduction (Table 2).
- **Day 8 of each cycle:** If eribulin cannot be administered on day 8, the dose should be delayed until recovery to above mentioned values (criteria for eribulin administration). The day 8 dose will be delayed for a maximum of 7 days and as follows:

- If the criteria for eribulin administration are met on, or before day 15 of a cycle, administer eribulin as recommended in Table 2; this dose is still called day 8. Eribulin administration on day 1of the next cycle must be no sooner than 14 days later.
- If the criteria for eribulin administration are NOT being met by day 15 of a cycle, omit the day 8 dose of eribulin for that cycle. Eribulin should be administered as recommended in Table 2 on day 1 of the next scheduled cycle assuming criteria to continue treatment are met within a maximum 2 week delay in therapy from originally planned day 8.

Table 1: Eribulin mesylate dose modification levels

Dose Level	Eribulin mesylate Dose
Starting Dose	1.4 mg/m^2
First dose reduction	1.1 mg/m^2
Second dose reduction	0.7 mg/m^2

Table 2: Dose modifications for toxicity – Eribulin mesylate

Toxicity	Grade and Details	Eribulin mesylate Dose Modifications
Neutropenia: (ANC= absolute	< 500 cells/mm3 lasting > 7 days in the previous cycle without use of growth factors	Hold eribulin until recovery to grade ≤1 and reduce by 1 dose level. Prophylactic growth factor support should be instituted for subsequent cycles.

neutrophil count)	< 500 cells/mm3 lasting </= 7 days in the previous cycle without use of growth factors	Hold eribulin until recovery to grade ≤1. Resume eribulin at the same dose with growth factor support.
	< 500 cells/mm3 lasting </= 7 days in the previous cycle despite use of growth factors	Hold eribulin until recovery to grade ≤1, and reduce by 1 dose level. Continue ongoing prophylactic growth factor support for subsequent cycles.
	<1000 /mm3 with fever or infection without use of growth factors in the previous cycle	Hold eribulin until recovery to grade ≤1. Resume eribulin at the same dose with growth factor support.
	<1000 /mm3 with fever or infection despite use of growth factors	Hold eribulin until recovery to grade ≤1, and reduce by 1 dose level, with prophylactic growth factor support.
Toxicity	**Grade and Details**	**Eribulin mesylate Dose Modifications**
	<1000 /mm3 without fever or infection	First occurrence: Hold eribulin until recovery to grade ≤1 then resume at the same dose with growth factor support. If uncomplicated neutropenia (<1000 /mm3 without fever or infection) occurs/recurs despite growth factor support, then hold eribulin until recovery to grade ≤1. Eribulin may be then be resumed at the same dose with growth factor support, or it may be reduced by one dose level, per investigator discretion. If a patient misses two consecutive Day 8 eribulin doses due to uncomplicated neutropenia, then hold eribulin until recovery to grade ≤ 1 and reduce by 1 dose level, and consider prophylactic growth factor support for subsequent cycles.
Platelets	Grade 3	Hold eribulin until recovery to grade ≤1 then reduce by 1 dose level.
	Grade 4	Hold eribulin until recovery to grade ≤1 then reduce by 1 dose level.
Anemia	Grade 4	Hold eribulin until recovery to grade ≤1 then reduce by 1 dose level. PRBC transfusions are permitted to treat anemia on protocol.

Non-hematologic toxicity	Grade 3, first occurrence	Hold eribulin until recovery to grade ≤1. Maximize supportive care measures. If symptom recovery occurs within 1 week, then eribulin may be resumed at the same dose if deemed appropriate by the investigator, otherwise, reduce by 1 dose level.
	Grade 3, despite maximal supportive measures	Hold eribulin until recovery to grade ≤1 then reduce by 1 dose level.
	Grade 4	Hold eribulin until recovery to grade ≤1 then reduce by 1 dose level.
*Participants requiring a delay of >2 weeks should go off protocol therapy. **Participants requiring > two dose reductions should go off protocol therapy.		
Growth factor support may be used instead of a dose reduction at the discretion of the investigator, as outlined above.		

6.2 Doxorubicin/cyclophosphamide (AC)

- The principle is to attempt to administer full doses of therapy on schedule.
- If AC is delayed due to toxicity and an investigator prefers drug administration every 3 weeks in order to maintain full dose, AC may be given on a 21 day cycle with approval of the Principal Investigator.
- No more than 1 dose-reduction of AC chemotherapy should be made for toxicity. If more than 1 dose-reduction would be required, AC should be discontinued, and the patient should proceed to surgery as outlined in the protocol (Cohort A) or proceed to eribulin as outlined in the protocol (Cohort B).
- If AC is held for > 2 weeks for toxicity, AC should be discontinued. Patients may proceed to surgery as outlined in the protocol (Cohort A) or proceed to eribulin (Cohort B).
- All dose modifications for AC are based on the dose level changes outlined below (Table 3).
- Instructions for management of toxicities related to AC are listed in Table 4. **Table 3: doxorubicin/cyclophosphamide (AC) – dose modification levels**

Dose Level	Doxorubicin dose	Cyclophosphamide dose
Starting dose	60 mg/m^2	600 mg/m^2
First dose reduction	45 mg/m^2	450 mg/m^2

Table 4: Dose Modifications for toxicity - doxorubicin/cyclophosphamide (AC)

Toxicity	Modifications for AEs that occur during a cycle but RESOLVE PRIOR TO THE NEXT TREATMENT CYCLE [a]	Modifications for AEs that REQUIRE A DELAY IN ADMINISTRATION OF THE TREATMENT CYCLE[b]
HEMATOLOGICAL:		

Diarrhea		
Grade 2 Grade 3 Grade 4	Maintain dose ↓ one dose level discontinue	↓ one dose level ↓ one dose level discontinue
Mucositis oral (stomatitis)		
Grade 2 Grade 3 Grade 4	Maintain dose ↓ one dose level discontinue	↓ one dose level ↓ one dose level discontinue
Vomiting *(despite antiemetics)*		
Grade 2 Grades 3, 4	↓ one dose level (optional) ↓ one dose level or discontinue	↓ one dose level discontinue
HEPATIC FUNCTION:		
Bilirubin or AST or ALP increased		
Neutrophils count decreased		
Grades 2, 3, 4	Maintain dose	*Hold until ≥ 1000/mm³ If recovery takes:* • 1 wk: maintain dose • 2 wks: ↓ one dose level
Febrile neutropenia	↓ one dose level or discontinue.	
HEMATOLOGICAL:		
Platelets		
Grades 2, 3 Grade 4	Maintain dose ↓ one dose level	*Hold until ≥ 100,000/mm³. If recovery takes:* • 1 wk: maintain dose • 2 wks: ↓ one dose level ↓ one dose level or discontinue.
GASTROINTESTINAL DISORDERS *(if related to chemotherapy, despite medical management)*:		

Grade 2	↓ one dose level	*Hold until bilirubin returns to the baseline grade, and AST and alkaline phosphatase have returned to≤ grade 1.* Then ↓ one dose level
Grade 3 Grade 4	↓ one dose level Discontinue	↓ one dose level Discontinue
OTHER CLINICALLY SIGNIFICANT AEs[c]:		
Grade 3 Grade 4	↓ one dose level discontinue	↓ one dose level Discontinue
*Dose modifications must be based on AEs that occur during the cycle (column 2) *and* AEs present on the scheduled cycle Day 1 (column 3). **Dose modifications must be based on the AE requiring the greatest modification.		

[a] Resolved means that all requiring dose modification are ≤ grade 1(except ANC [which must be ≥ 1000/mm3] and bilirubin [which must be ≤ the baseline grade]) on Day 1 of the next scheduled cycle of AC (ie, treatment can be given without delay).
[b] Hold and check weekly. With exception of ANC and bilirubin, resume treatment when toxicity is ≤ grade 1. If toxicity has not resolved after 2 weeks of delay, discontinue doxorubicin/cyclophosphamide. [c] Determination of "clinically significant" AEs is at the discretion of the investigator.

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of reported and/or potential AEs (Section 7.1) and the characteristics of an observed AE (Section 7.2) will determine whether the event requires expedited reporting **in addition** to routine reporting.

7.1 Expected Toxicities

Most AEs on studies with eribulin are grade 1 or 2. Peripheral neuropathy was an important AE leading to discontinuation of therapy. The most frequently reported eribulin mesylate related AEs were asthenia/fatigue (65%), alopecia (60%), neutropenia (60%), nausea (44%), anemia (28%), pyrexia (23%), leucopenia (22%), anorexia (21%), constipation (19%), vomiting (18%), and peripheral neuropathy (only grade 3: 5.5%). Grade 4 neutropenia occurred in 32% of patients, and fever with neutropenia occurred in 5.5% of patients. The frequency of all other grade 3/4 AEs was less than 3%.[31]

Adverse Events List(s)

Refer to the Investigator's Brochure for detailed eribulin mesylate information and FDA approved package insert for eribulin mesylate for more information.

Adverse Event List(s) for eribulin mesylate

AE of special interest of eribulin are:
- Hematological toxicity,
- Asthenia or fatigue,
- Alopecia,
- Nausea,
- Peripheral neuropathy.

Adverse Event List(s) for Other Agent(s)

Please refer to the FDA approved doxorubicin and cyclophosphamide package inserts for the comprehensive list of adverse events.

AE of special interest for doxorubicin include:
- Hematological toxicity,
- Asthenia or fatigue,
- Alopecia,
- Nausea,
- Cardiac toxicity,
- Mucositis,
- Secondary leukemia.

AE of special interest for cyclophosphamide include:
- Hematological toxicity,
- Asthenia or fatigue,
- Alopecia,
- Nausea, • Hemorrhagic cystitis.

7.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **For expedited reporting purposes only:**
  - AEs for the agent(s) that are listed above should be reported only if the adverse event varies in nature, intensity or frequency from the expected toxicity information which is provided.
  - Other AEs for the protocol that do not require expedited reporting are outlined in the next section (Expedited Adverse Event Reporting) under the sub-heading of ProtocolSpecific Expedited Adverse Event Reporting Exclusions.

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

7.3 Expedited Adverse Event Reporting

Investigators **must** report to the Overall PI any serious adverse event (SAE) that occurs after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment on the local institutional SAE form.

DF/HCC Expedited Reporting Guidelines

Investigative sites within DF/HCC and DF/PCC will report SAEs directly to the DFCI Office for Human Research Studies (OHRS) per the DFCI IRB reporting policy. .

7.4 Reporting Serious Adverse Events to [redacted]

Serious adverse events (SAEs), as defined below, where the Overall PI considers a relationship to the eribulin therapy to be at least a reasonable possibility, will be reported to [redacted] on a Medwatch 3500A form within one business day of the event. Serious adverse events (SAEs) are not related to eribulin therapy and non-serious AEs will be provided to [redacted] in the final study report and any interim reports provided.

A serious adverse event (SAE) is any adverse event, occurring at any dose and regardless of causality that:

- Results in death
- Is life-threatening. Life-threatening means that the person was at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction which hypothetically might have caused death had it occurred in a more severe form.

- Requires or prolongs inpatient hospitalization (i.e., the event required at least a 24-hour hospitalization or prolonged a hospitalization beyond the expected length of stay). Hospitalization admissions and/or surgical operations scheduled to occur during the study period, but planned prior to study entry are not considered SAEs if the illness or disease existed before the person was enrolled in the trial, provided that it did not deteriorate in an unexpected manner during the trial (e.g., surgery performed earlier than planned).
- Results in persistent or significant disability/incapacity. Disability is defined as a substantial disruption of a person's ability to conduct normal life functions.
- Is a congenital anomaly or birth defect; or
- Is an important medical event when, based upon appropriate medical judgment, it may jeopardize the participant and require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home; blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Events **not** considered to be serious adverse events are hospitalizations for:

- routine treatment or monitoring of the studied indication, not associated with any deterioration in condition, or for elective procedures
- elective or pre-planned treatment for a pre-existing condition that did not worsen
- emergency outpatient treatment for an event not fulfilling the serious criteria outlined above and not resulting in inpatient admission
- respite care

The reports will be sent on MedWatch 3500A form to [redacted] at the number listed below:

[redacted] safety fax number [redacted] Email: [redacted]

7.5 Expedited Reporting to Hospital Risk Management

Participating investigators will report to their local Risk Management office any participant safety reports or sentinel events that require reporting according to institutional policy.

7.6 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions to the Overall PI on the toxicity case report forms. **AEs reported through expedited processes (e.g., reported to the IRB, FDA, etc.) must <u>also</u> be reported in routine study data submissions.**

8. PHARMACEUTICAL INFORMATION

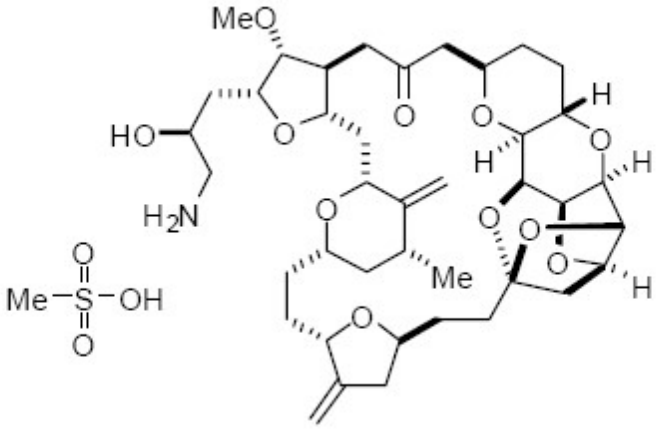
A list of the adverse events and potential risks associated with the investigational or other agents administered in this study can be found in Section 7.1.

8.1 Eribulin mesylate

Refer to the Investigator's Brochure for detailed agent information and eribulin mesylate FDA approved package for more information.

Description

Chemical name: 11,15:18,21:24,28- Triepoxy-7,9-ethano-12,15-methano-9H,15Hfuro[3,2-i]furo[2',3':5,6]pyrano[4,3-*b][1,4]dioxacyclopentacosin-5(4H)-one,*2-[(2S)-3amino-2-hydroxypropyl]hexacosahydro-3-methoxy-26-methyl-20,27-bis(methylene)(2R,3R,3aS,7R,8aS,9S,10aR,11S,12R,13aR,13bS,15S,18S,21S,24S,26R,28R,29aS)-, methanesulfonate (salt)
Empirical formula: C40H59NO11•CH4O3S **Molecular weight:** 826.0 (729.9 for free base). **Structural formula:**



Other name: E7389, Halaven®
Mode of action: Eribulin injection is a non-taxane microtubule dynamics inhibitor.
Manufacturer: [redacted]
Molecular weight: 826.0 (729.9 for free base).
Distribution: Vd: 43-114 L/m2
Protein binding: 49% to 65% **Metabolism:** Negligible **Half-life, elimination:** ~40 hours
Excretion: Feces (82%; predominantly as unchanged drug); urine (9%, primarily as unchanged drug)

Form

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

Storage and Stability

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.

Compatibility

Eribulin is compatible with saline (0.9% Sodium Chloride Injection). It is not compatible with solutions with dextrose.

Handling

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of the chemotherapeutic agent in a self-contained and protective environment.

Availability

Eribulin mesylate is a commercially available agent but will be supplied free-of-charge from Eisai.

Preparation

The amount of eribulin required will be withdrawn from the appropriate number of vials into a syringe. This may be injected directly as an i.v. infusion over approximately 2 to 5 minutes or diluted in up to 100 mL 0.9% saline for i.v. infusion over approximately 2 to 5 minutes. 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.

Administration

It will be administered over approximately 2-5 minutes. Do not administer in the same intravenous line concurrent with the other medicinal products.

Ordering

Eribulin will be provided by [redacted] and stored in the site research pharmacy. Under no circumstances will the investigator allow the study treatment(s) to be used other than as directed by this protocol. Clinical supplies will not be dispensed to any individual who is not enrolled in the study.

Accountability

The investigator, or a responsible party designated by the investigator, should maintain a careful record of the inventory and disposition of the agent using the NCI Drug Accountability Record Form (DARF) or another comparable drug accountability form. (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.)

Destruction and Return

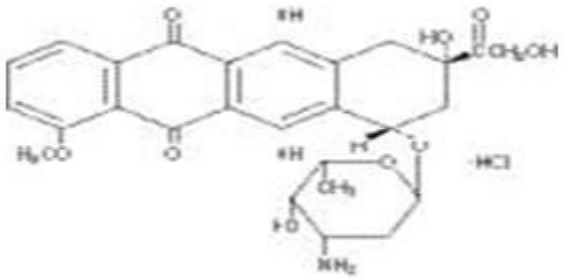
At the end of the study, unused supplies of eribulin should be destroyed according to institutional policies. Destruction will be documented in the Drug Accountability Record Form.

8.2 Doxorubicin

Refer to the FDA approved package description for more information.

Description

Doxorubicin is a cytotoxic anthracycline antibiotic isolated from cultures of Streptomyces peucetius var.caesius. Doxorubicin consists of a naphthacenequinone nucleus linked through a glycosidic bond at ring atom 7 to an amino sugar, daunosamine. Chemically, doxorubicin hydrochloride is (8S,10S)-10-[(3-Amino-2,3,6-trideoxy-a-Llyxo-hexopyranosyl)-oxy]-8-glycoloyl-7,8,9,10-tetrahydro-6,8,11-trihydroxy-1-methoxy5,12-naphthacenedione hydrochloride. The structural formula is as follows:



Doxorubicin binds to nucleic acids, presumably by specific intercalation of the planar anthracycline nucleus with the DNA double helix. The anthracycline ring is lipophilic, but the saturated end of the ring system contains abundant hydroxyl groups adjacent to the amino sugar, producing a hydrophilic center. The molecule is amphoteric, containing acidic functions in the ring phenolic groups and a basic function in the sugar amino group. It binds to cell membranes as well as plasma proteins.

The cytotoxic effect of doxorubicin on malignant cells and its toxic effects on various organs are thought to be related to nucleotide base intercalation and cell membrane lipid binding activities of doxorubicin. Intercalation inhibits nucleotide replication and action of DNA and RNA polymerases. The interaction of doxorubicin with topoisomerase II to form DNA-cleavable complexes appears to be an important mechanism of doxorubicin cytocidal activity.

Doxorubicin cellular membrane binding may affect a variety of cellular functions. Enzymatic electron reduction of doxorubicin by a variety of oxidases, reductases and dehydrogenases generates highly reactive species including the hydroxyl free radical OH•. Free radical formation has been implicated in doxorubicin cardiotoxicity by means of Cu (II) and Fe (III) reduction at the cellular level.

Pharmacokinetics
Pharmacokinetic studies, determined in patients with various types of tumors undergoing either single or multi-agent therapy have shown that doxorubicin follows a multiphasic disposition after intravenous injection. In four patients, doxorubicin has demonstrated dose-independent pharmacokinetics in the dose range of 30 to 70 mg/m2.

Distribution
The initial distribution half-life of approximately 5 minutes suggests rapid tissue uptake of doxorubicin, while its slow elimination from tissues is reflected by a terminal half-life of 20 to 48 hours. Steady-state distribution volume ranges from 809 to 1214 L/m2 and is indicative of extensive drug uptake into tissues. Binding of doxorubicin and its major

metabolite, doxorubicinol, to plasma proteins is about 74 to 76% and is independent of plasma concentration of doxorubicin up to 1.1 mcg/mL.

Metabolism
Enzymatic reduction at the 7 position and cleavage of the daunosamine sugar yields aglycones which are accompanied by free radical formation, the local production of which may contribute to the cardiotoxic activity of doxorubicin. Disposition of doxorubicinol (DOX-OL) in patients is formation rate limited, with the terminal half-life of DOX-OL being similar to doxorubicin. The relative exposure of DOX-OL, i.e., the ratio between the AUC of DOX-OL and the AUC of doxorubicin, compared to doxorubicin ranges between 0.4 and 0.6.

Excretion
Plasma clearance is in the range 324 to 809 mL/min/m2 and is predominately by metabolism and biliary excretion. Approximately 40% of the dose appears in the bile in 5 days, while only 5 to 12% of the drug and its metabolites appear in the urine during the same time period. In urine, <3% of the dose was recovered as DOX-OL over 7 days.

Form

Commercially available from various manufacturers as lyophilized powder for reconstitution in 10-, 20-, 50-, 100-and 150-mg vials. Also available as solution (2 mg/ml) in 10-, 20-, 50- and 200-mg vials for injection. Please refer to FDA-approved package insert for complete product information.

Storage and Stability

Intact vials of doxorubicin should be stored in the refrigerator. Intact vials of powder for reconstitution should be stored at room temperature. Reconstituted solutions are stable for 7 days at room temperature and 15 days under refrigeration when protected from light. Commercially available solutions labeled as such are intended to be multi-dose vials.

Compatibility

Doxorubicin is compatible with saline (0.9% Sodium Chloride Injection). Doxorubicin should not be mixed with heparin or fluorouracil since it has been reported that these drugs are incompatible to the extent that a precipitate may form. Contact with alkaline solutions should be avoided since this can lead to hydrolysis of doxorubicin. Until specific compatibility data are available, it is not recommended that doxorubicin be mixed with other drugs.

Handling

Procedures for proper handling and disposal of anti-cancer drugs should be considered. Several guidelines on this subject have been published. There is no general agreement that all the procedures recommended in the guidelines are necessary or appropriate. However, given the toxic nature of this substance, the following protective recommendations are provided:

Personnel should be trained in good technique for handling.

Pregnant staff should be excluded from working with this drug.

Personnel handling doxorubicin should wear protective clothing: goggles, gowns and disposable gloves and masks.

A designated area should be defined and the work surface should be protected by disposable, plastic-backed, absorbent paper.

All items used for administration or cleaning, including gloves, should be placed in high-risk waste-disposal bags for high-temperature incineration.

Spillage or leakage should be treated with dilute sodium hypochlorite (1% available chlorine) solution, preferably by soaking, and then water.

All cleaning materials should be disposed of as indicated previously.

In case of skin contact thoroughly wash the affected area with soap and water or sodium bicarbonate solution. However, do not abrade the skin by using a scrub brush.

In case of contact with the eye(s), hold back the eyelid(s) and flush the affected eye(s) with copious amounts of water for at least 15 minutes. Then seek medical evaluation by a physician.

Always wash hands after removing gloves.

Availability

Commercially available from various manufacturers.

Preparation

Reconstitute the vials with 5, 10, 25, 50, or 75 ml, respectively, of sodium chloride for injection, USP.

Administration

Administer doxorubicin intravenously, either peripherally as a bolus injection or through a central venous line. Avoid extravasation, since severe local tissue necrosis may result. Administration time is per institutional standards.

Ordering

Doxorubicin will be ordered by pharmacy as institutional policies.

Accountability

The investigator, or a responsible party designated by the investigator, should maintain a careful record of the inventory and disposition of the agent using the NCI Drug Accountability Record Form (DARF) or another comparable drug accountability form. (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.)

Destruction and Return

Unused supplies of doxorubicin should be destroyed according to institutional policies.

8.3 Cyclophosphamide

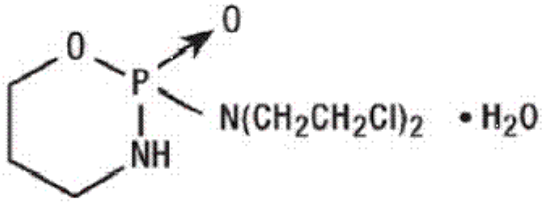
Refer to the FDA approved package description for more information.

Description

Cyclophosphamide (INN, trade names
Endoxan, Cytoxan, Neosar, Procytox, Revimmune), also known as cytophosphane, is a nitrogen mustard alkylating agent from the oxazaphosphorine group. An alkylating agent adds an alkyl group to DNA. It attaches the alkyl group to the guanine base of DNA, at the number 7 nitrogen atom of the imidazole ring. This interferes with DNA replication by forming intrastrand and interstrand DNA crosslinks.

Cyclophosphamide for Injection, USP is a sterile white powder containing cyclophosphamide monohydrate. Cyclophosphamide is a synthetic antineoplastic drug chemically related to the nitrogen mustards. Cyclophosphamide is a white crystalline powder with the molecular formula C7H15CI2N2O2P•H2O and a molecular weight of 279.1. The chemical name for cyclophosphamide is 2-[bis(2chloroethyl)amino]tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide monohydrate.

Cyclophosphamide is soluble in water, saline, or ethanol and has the following structural formula:



Pharmacokinetics
Cyclophosphamide is administered as an inactive prodrug that must undergo activation to form the active metabolite 4 hydroxy cyclophosphamide through phase I metabolism by cytochrome P450 (CYP) enzymes 2B6, 2C8, 2C9, 2C19, 3A4 and 3A5. Detoxification is primarily through glutathione S transferases (GSTA1, GSTP1) and alcohol dehydrogenase (ALDH1, ALDH3). Concomitant therapy with inducers of cyclophosphamide metabolizing enzymes (e.g. CYP 2B6, 2C9, 3A4) enhances the enzyme expression and may potentially increase the formation of metabolites responsible for cytotoxicity. In contrast, the inhibitors could interfere with cyclophosphamide activation and may alter the effectiveness of cyclophosphamide treatment.

The decline in CP plasma levels following an i.v. dose is biexponential with terminal half-life averaging 7 hours (1.8 to 12.4) for adults, and 4 hours (2.4 to 6.5) for children; daily administration of approximately 50 mg/Kg bid or qid (i.v. infusion) to children significantly decreased both plasma half life and urinary excretion of CP. With daily exposure or repeated high-dose administration (i.v.) of cyclophosphamide to adult patients, the half-life of CP decreased without an increase in urinary excretion, suggesting that the drug induces its own metabolism. After an i.v. dose, the NBP [4-(nitrobenzyl)pyridine] plasma alkylating activity peaks 2 hours after administration, and declines with a half-life of 7.7 hours. Phosphoramide mustard in 3 patients, receiving 60-75 mg/Kg cyclophosphamide, peaked 2 to 3 hours after the administration of CP at levels 10 to 20% of the unchanged drug, and declined slowly with levels still detectable at 24 hours. Even with doses as high as 80 mg/Kg, the plasma half-life of CP does not increase.

The t½ and AUC of cyclophosphamide after a 5-day continuous infusion schedule of 300-400 mg/m2/day, were similar to the t½ and AUC of a 1500 mg/m2 i.v. bolus. The AUC of the alkylating activity after 5-day i.v. infusion, however, was three times higher than the AUC of alkylating activity after 1500 mg/m2 i.v. bolus administration of cyclophosphamide.

After CP administration to man and laboratory animals, significant differences in the pharmacokinetic parameters of the active metabolite 4-hydroxycyclophosphamide in

both man and animals were found. In man, the active metabolite in blood was found at only low but longer lasting concentrations compared to the high and relatively short time concentration in blood of mice and rats, after a comparable dose.

Distribution
A mean apparent volume of distribution of cyclophosphamide was 0.56 L/Kg in adults and 0.67 L/Kg in children. Tissue Distribution of CP after i.v. administration to cancer patients indicated that both unchanged parent drug and metabolites in small quantities penetrate the blood brain barrier; brain tissue concentrations being similar to those in blood. Biopsies, performed 2 hours after CP infusion, indicated approximately 30% more radioactivity in lymph nodes compared to muscle, adipose tissue or skin, but relative proportions of unchanged drug metabolites were not established.

Protein Binding: 12 to 14% of unchanged cyclophosphamide is protein-bound; the alkylating metabolites, however, are more extensively bound, namely 67% of the total plasma alkylating activity, and in another study, 39% of phosphamide mustard was protein-bound.

Metabolism
While chemically not reactive, the primary metabolites 4-hydroxycyclophosphamide and aldophosphamide are cytotoxic in vitro, and may represent transport forms of the alkylating moiety, phosphoramide mustard. The two primary metabolites can be further oxidized into the major urinary metabolites 5-ketocyclophosphamide and carboxyphosphamide. Nor-nitrogen mustard, a decomposition product of carboxyphosphamide, is an active alkylating agent with cytotoxicity in vivo and in vitro, however, little antitumour activity could be demonstrated; yet, it may play a role in the hematopoietic and other toxicities of cyclophosphamide. Another metabolite formed from aldophosphamide is acrolein, which has been identified as the most urotoxic species.

Excretion
In man, a generally higher proportion of the administered CP is excreted as metabolites in urine. Urinary recovery of radioactivity after intravenously administered ^{14}Ccyclophosphamide to patients ranged from 59 to 82% after 4 days, while not more than 20% of i.v. cyclophosphamide was excreted unchanged in urine at any dose level.

Renal clearance estimates of between 5.3 and 11 mL/min indicate substantial renal tubular reabsorption.

Form

Commercially available as powder for injection in 100-mg, 200-mg, 500-mg, 1-g, and 2g vials. Please refer to the FDA-approved package insert for complete product information.

Storage and Stability

Store intact vials of powder at room temperature (15° to 30°C). Reconstitute lyophilized cyclophosphamide is chemically and physically stable for 24 hours at room temperature or for 6 days in the refrigerator (2° to 8°C). It does not contain any antimicrobial preservative, and care must therefore be taken to ensure the sterility of prepared solution.

Compatibility

It is prudent to monitor, among others, the following drugs if administered concurrent with cyclophosphamide: Colchicine, Probenecid, Sulfinpyrazone, Chlorambucil, Mercaptopurine. PROCYTOX should not be reconstituted with benzyl alcohol-preserved diluent solution such as bacteriostatic sodium chloride when used in children or infants, due to toxicity concerns in this age group (i.e., gasping syndrome in infants). Further, PROCYTOX should not be reconstituted or diluted with benzyl alcohol-containing diluents, as benzyl alcohol may catalyse the decomposition of cyclophosphamide. Therefore, it is recommended to reconstitute PROCYTOX with isotonic, sterile, Sodium Chloride Injection USP.

Handling

Preparation of PROCYTOX must take place in a Pharmacy or, in facilities where there is not a Pharmacy, in a Class II Type B or better, externally-vented biological safety cabinet. The biological safety cabinet should have airflow monitoring devices and should be certified at least annually. Only luer-lock connections should be used in the preparation of PROCYTOX.

Procedures for proper handling and disposal of anti-cancer drugs should be considered. Several guidelines on this subject have been published. There is no general agreement that all the procedures recommended in the guidelines are necessary or appropriate. However, given the toxic nature of this substance, the following protective recommendations are provided:

Personnel should be trained in good technique for handling.

Pregnant staff should be excluded from working with this drug.

Personnel handling doxorubicin should wear protective clothing: goggles, gowns and disposable gloves and masks.

A designated area should be defined and the work surface should be protected by disposable, plastic-backed, absorbent paper.

All items used for administration or cleaning, including gloves, should be placed in high-risk waste-disposal bags for high-temperature incineration.

Spillage or leakage should be treated with dilute sodium hypochlorite (1% available chlorine) solution, preferably by soaking, and then water.

All cleaning materials should be disposed of as indicated previously.

In case of skin contact thoroughly wash the affected area with soap and water or sodium bicarbonate solution. However, do not abrade the skin by using a scrub brush.

In case of contact with the eye(s), hold back the eyelid(s) and flush the affected eye(s) with copious amounts of water for at least 15 minutes. Then seek medical evaluation by a physician.

Always wash hands after removing gloves.

Availability

Commercially available from various manufacturers.

Preparation

Reconstitute 100-mg, 200-mg, 500-mg, 1-g, and 2-g vials with 5,10, 25, 50, or 100 ml of sterile water for injection respectively, for a final concentration of 20 mg/ml. Vigorous shaking an/or gentle warming may be necessary. Bacteriostatic water (paraben-preserved only) may also be used for reconstitution. 0.9% sodium chloride or 5% dextrose may also be used for reconstitution.

Administration

Intravenous administration preferably should be conducted as an infusion. To reduce the likelihood of adverse reactions that appear to be administration rate-dependent (e.g., facial swelling, headache, nasal congestion, scalp burning), cyclophosphamide should be injected or infused very slowly. Duration of the infusion also should be appropriate for the volume and type of carrier fluid to be infused.

Ordering

Cyclophosphamide will be ordered by pharmacy as institutional policies.

Accountability

The investigator, or a responsible party designated by the investigator, should maintain a careful record of the inventory and disposition of the agent using the NCI Drug Accountability Record Form (DARF) or another comparable drug accountability form. (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.)

Destruction and Return

Disposal of cyclophosphamide-contaminated clothing, gloves, utensils, broken glass etc. must be considered as hazardous waste. It must be deposited into a 4 mil thick polypropylene hospital trash bag (properly labeled), or be otherwise segregated and incinerated at above 1000 C. Chemical inactivation should, if possible, be avoided, since it is often ineffective and may produce byproducts that are more mutagenic than the parent drug. Unused supplies of cyclophosphamide should be destroyed according to institutional policies.

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES (COHORT A AND B)

9.1 Biomarker Studies

The study also requires mandatory plasma/serum samples for biomarker research, which may include, among other analyses, the assessment of circulating tumor DNA. Additional candidate markers of response to treatments that emerge from other clinical or nonclinical studies may also be assessed in this study and can be assessed with different types of technologies.

Handling of Samples:

Research blood collection is mandatory for all patients for DNA isolation and cell-free circulating DNA analysis. The samples will be banked in the DF/HCC Core and Blood Tissue Bank in order to extract germline and cell-free DNA to be used as normal DNA reference for tumor tissue-based studies and for future research purposes. These specimens will become the property of the DF/HCC.

The following research blood samples are required:

- Two 10 mL lavender top (EDTA Fisher #366643) tubes of whole blood at baseline (or at any time after registration but before initiating study therapy).

- Two 10 mL Streck blood collection tubes will be collected at baseline, prior to the first dose of AC (Cycle 5 Day 1) for Cohort A and prior to the first dose of Eribulin (Cycle 5 Day 1) for Cohort B, the presurgery visit, and the post-surgical visit (8 tubes total).

All samples should be de-identified and labeled with the Participant initials, Participant Study ID number and date of collection and time point (e.g., "Baseline" or "Presurgery"). Processing instructions for whole blood and circulating cfDNA are provided in the table below.

Sample Type	Processing Instructions
Lavender Top Tubes: Whole Blood for genomic analysis	Collect 20 cc of whole blood by standard venous phlebotomy into the EDTA (purple top) tubes provided. Tube should be labeled with patient study number, patient initials, and date /time of collection. After collection, invert the tubes 10 times to ensure adequate mixing and anticoagulation. DO NOT centrifuge this tube. Store tube at room temperature until ready for shipment. Ship in the ambient temperature (cold pack) compartment of the shipping kit. Do not freeze.
Streck Tubes: Circulating cfDNA	Collect 20 mL of whole blood into 2 x 10 ml Streck BCT tubes Invert tubes 10 times. Streck tubes should be kept at 6°C (42.8°F) to an ambient temperature of 37°C (98°F) until shipping. Streck tubes should be kept at 6°C (42.8°F) to an ambient temperature of 37°C (98°F) during shipping. Do not freeze specimens.

DO NOT FREEZE OR REFRIGERATE STRECK TUBES. Streck tubes must be stored at room temperature to preserve the sample.

Please email the DFCI Core Lab with the sample information the prior to the scheduled blood draw at: [redacted]

9.2 Laboratory Correlative Studies

Reversion of EMT phenotype (MET) and Normalization of the tumor vessel phenotype

Collection and handling of specimens is the same for both cohorts.

Collection of Specimen(s)

A core needle breast tumor biopsy (14 gauge) will be acquired from all participants prior to starting protocol therapy, and on cycle 1 day 8 of treatment. Any residual tumor present in the mastectomy specimen will also be obtained. Research breast biopsies (baseline and cycle 1 day 8) are mandatory.

Handling of Specimens(s)

For research breast biopsies, 6-8 cores will be collected per timepoint (2 FFPE, 4 OCT). Two cores will be immediately formalin fixed before being embedded in paraffin blocks and stored. Four cores will be embedded in OCT and then immediately frozen on dry ice and stored at -80°C.

For any mastectomy tissue, the tumor section size should be about 20 mg. Only residual tumor measuring ≥ 1 cm found within the mastectomy specimen on gross examination will be collected. Half of the specimen will be frozen on dry ice and stored at -80°C and half will be immediately formalin-fixed before being embedded in paraffin blocks and stored. Tumor tissue frozen in OCT may be stored at -80°C indefinitely.

Coded laboratory specimens will be stored in the Tumor Bank of the DFCI. These specimens will become the property of DFCI. Patients will be informed that their specimens may be used for research by investigators at DF/HCC.

9.3 Special Studies

Reversion of EMT phenotype (MET) and Normalization of the tumor vessel phenotype

Outcome Measure

Preclinical data suggests that eribulin induces two specific biological effects in breast cancers: Reversion of EMT phenotype (MET) [25, 32] and normalization of the tumor vessel phenotype [29, 33]. Exploratory studies will be performed during this trial to determine if similar changes are seen after eribulin treatment of human inflammatory breast cancer. Identical exploratory studies will be performed after exposure to AC to determine if the biological effects are specific to eribulin, or due to chemotherapy in general.

Method of Assessment

- RNA extracted from biopsy specimens with >50% tumor cellularity
- Reverse transcription to cDNA using standard protocols

- Expression of 10 EMT-related genes determined in each sample by RT-qPCR
- TGFB1, TGFB2, CDH1, KRT18, CDH2, VIM, TWIST1, SNAI2, ZEB1, ZEB2

- Primers designed in-house (PrimerBlast)
- 2 housekeeping genes included

- Expression of angiogenesis-related genes determined in each sample by RT-qPCR
- 15 genes selected based on pre-clinical data. Each has a defined role in modulating vessel phenotype or EC-PVC interactions
- *VEGFA, VEGFR1, VEGFR2, VEGFR3, PGF, DLL4, JAG1, NOTCH4, EFNB2, EPHA2, EPHB1, WNT5A, WNT11, BPM4, CA9.*

Timing of Assessment

- Prior to starting protocol therapy and on Cycle 1, day 8
- Residual tumor harvested from mastectomy specimen.

Method of Data Recording

All RT-qPCR data (raw data) will be stored in an electronic database within the Department of Cancer Biology, Dana-Farber Cancer Institute. Data will be de-identified to clinical details or patient demographics. The database will also be copied and maintained on a second storage drive also held within the Department of Cancer Biology.

9.3.6 Timing of Data Recording

- RT-qPCR for both cohorts will be conducted at the completion of the study, and data stored immediately as described.

9.4 Imaging Sub-study

9.4.1 Eribulin and vessel normalization

- This analysis will be performed on a sub-set of the patient population (N=5 from Cohort A; N=5 from Cohort B)

9.4.2 Outcome Measure

Pre-clinical data demonstrated vascular remodeling among MX-1 and MDA-MB-231 human breast cancer xenograft models 5 to 6 days after a single exposure to eribulin. [29] The K^{trans} values from tumor rim and core regions following treatment with eribulin resulted in a significant increase of K^{trans} in tumor cores by day 6. The iAUC histogram analysis of the MX-1 xenograft model treated with eribulin demonstrated a rightward shift of the curve resulting in a convergence of the curves for tumor and rim between days 6 and 10. These results suggest that eribulin treatment induces tumor vascular remodeling which may contribute to its efficacy in human cancers. Exploratory studies

will be performed during this trial to determine if similar changes are seen after eribulin treatment of human inflammatory breast cancer. Identical studies will be performed after exposure to AC to determine if these vascular effects are specific to eribulin, or due to chemotherapy in general.

9.4.3 Method of Assessment

- Patients will undergo a baseline DCE-MRI of the affected breast, and then receive their first therapeutic dose of eribulin (Cohort A) or AC (Cohort B). Approximately 6-8 days later, DCE-MRI of the affected breast is repeated on the same scanner (Siemens 3T scanner).

- Changes in K^{trans}, v_e and v_p determination of the tumor core and rim will be analyzed, as will changes in the iAUC computed pre-treatment and 6-8 days post-eribulin (Cohort A) or AC (Cohort B) dosing.

9.4.4 Timing of Assessment

- Imaging must be done after the research breast biopsy scheduled at baseline (prior to starting protocol therapy) and before cycle 1, day 8 research biopsy (after one dose of protocol therapy).

9.4.5 Method of Data Recording

- DCE MRI Acquisition.

  DCE MRI will be performed at the Dana-Farber Cancer Institute using a Siemens 3T scanner with a breast coil. Dynamic scan will be acquired prior to and after contrast agent gadopentetate dimeglumine, Gd- DTPA, is injected intravenously via a power injector.

- Quantify K^{trans}, Normalized vessel area.

  The semiquantitative parameter, iAUC, and the quantitative physiological parameters,
  K^{trans}, v_e and v_p, will be calculated for all tumor voxels from the DCE-MRI data.[34]

- Compare peripheral rim of tumor and central core of tumor.
  Regions-of-Interest (ROIs) will be manually drawn on the K^{trans} map to select either the core or the rim of the lesion. The ROIs will be copied from the K^{trans} map to the v_e, v_p and iAUC maps. Mean and histogram analysis in these ROIs will be compared between lesion rim and core.

9.4.6 Timing of Data Recording

- Data recording of each DCE-MRI scan will be performed shortly after acquisition.

10. STUDY CALENDAR

Baseline evaluations are to be conducted within 4 weeks (28 days) prior to start of protocol therapy unless otherwise noted. Baseline imaging may be performed up to 35 days prior to the start of protocol therapy. .

Assessments must be performed prior to administration of any study agent. Study assessments and agents should be administered within ± 2 days of the protocol-specified date, unless otherwise noted.

Cohort A:

	Screening (≤ -28 days prior to treatment)	Pre-operative therapy			Pre-surgical evaluation	Surgery[k]	Post-surgical evaluation	Follow-up Visits[m]
		Cycle 1-4, eribulin day 1	Cycle 1-4, eribulin day 8	Cycle 5-8, AC day 1				
Eribulin mesylate		A	A					
Doxorubicin / cyclophosphamide (AC)				A				
Concurrent meds	X	X		X	X		X	
Physical exam[a,]	X	X		X	X		X	
Clinical tumor (breast/lymph nodes) assessment[a]	X	X		X	X			

Vital signs[b]	X	X	X	X	X		X	
Performance status	X	X		X	X		X	
CBC w/diff, plts	X[c]	X	X	X	X		X	

Serum chemistry[d]	X[c]	X	X	X	X		X	
Magnesium		X[e]						
EKG (12-lead)	X							
Adverse event evaluation		X		X	X		X	
Pregnancy Test[f]	X							
Mammogram/ ultrasound	X				X[h]			X[i]
PET/CT	X							
CT chest, abdomen, pelvis	X							
Clinical Breast MRI	X				X[h]			
Cardiac Echo[g]	X							
Mastectomy[j]						X		
Radiation therapy[k]								X
Endocrine therapy[k]								X

Research breast tissue collection[l]	X		X			X		
DCE-MRI[n]	X		X					
Research blood collection[o]		X		X	X		X	

A: Eribulin mesylate – 1.4mg/m^2 IV day 1, 8 every 21 days x 4 cycles. One cycle = 21 days.

B: AC – doxorubicin 60mg/m^2 IV and cyclophosphamide 600mg/m^2 IV day 1 every 14 days x 4 cycles. One cycle = 14 days. Begin AC 2 weeks (14 days +/2 days) after cycle 4, day 8 of eribulin.

a: Physical examination performed on Day 1 of every cycle (+/- 2 days). Physical examination must be performed by a medically qualified individual such as a licensed physician, physician's assistant, or advanced registered nurse practitioner. b. Vital signs should include: BP, HR, respiratory rate, body temperature and weight. Height performed at baseline only. c: If screening labs done ≤ 7 days of C1D1, they do not need to be repeated

d: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.

e: Magnesium only required at C1D1 f: Serum or urine pregnancy test (women of childbearing potential). g: ECHO is preferred but MUGA will be accepted. h: Breast imaging must be done 1-2 weeks after completing preoperative treatment, prior to surgery. i: Breast imaging per standard practice recommendations. j: Surgery to occur 3 to 5 weeks following completion of AC.

k: Radiation therapy to begin approximately 3 to 6 weeks following surgery. Endocrine therapy (for ER and/or PR positive disease) per institutional standards may begin concurrent with or after completion of radiation therapy per physician preference.

l: Breast biopsy performed at baseline and prior to cycle 1, day 8. Research biopsies can be done the same day as treatment. Baseline biopsy can be done any time prior to initiating treatment, cycle 1, day 1. Cycle 1 day 8 biopsies can be done within 1 day prior to therapy. Tissue is also to be collected at the time of surgery. See section 9.2 for tissue collection guidelines.

m: Every 3 months (+/- 1 month) x 1 year, then every 6 months (+/- 1 month) x 4 years, then annually. Patients can be evaluated at any time as deemed clinically necessary by the investigator.

n: DCE-MRI performed in 5 patients. Imaging performed at baseline and prior to cycle 1, day 8. Baseline imaging can be performed any time prior to initiating treatment (cycle 1, day 1) but imaging must be performed after the research breast biopsy at baseline and the second DCEMRI must be performed prior to the cycle 1, day 8 research biopsy. DCE-MRI of the affected breast is repeated on the same scanner (Siemens 3T scanner). There must be between 5-8 days between both DCE-MRI scans.

o: Research blood samples collected at screening OR C1D1, C5D1, pre-surgical evaluation and post-surgical evaluation. See Section 9.1 for details.

Cohort B:

	Screening (≤ -28 days prior to treatment)	Pre-operative therapy				Pre-surgical evaluation	Surgery[k]	Post-surgical evaluation	Follow-up Visits[m]
		Cycle 1-4, AC day 1	Cycle 1 day 8	Cycle 5-8, eribulin day 1	Cycle 5-8, eribulin day 8				
Eribulin mesylate				B	B				
Doxorubicin / cyclophosphamide (AC)		B							
Concurrent meds	X	X		X		X		X	
Physical exam[a,]	X	X		X		X		X	
Clinical tumor (breast/lymph nodes) assessment[a]	X	X		X		X			
Vital signs[b]	X	X		X	X	X		X	
Performance status	X	X		X		X		X	
CBC w/diff, plts	X[c]	X		X	X	X		X	
Serum chemistry[d]	X[c]	X		X	X	X		X	
Magnesium				X[e]					
EKG (12-lead)	X								
Adverse event evaluation		X		X		X		X	

B-HCG[f]	X								
Mammogram/ ultrasound	X					X[h]			X[i]

PET/CT	X								
CT chest, abdomen, pelvis	X								
Clinical Breast MRI	X					X[h]			
Cardiac Echo[g]	X								
Mastectomy[j]							X		
Radiation therapy[k]									X
Endocrine therapy[k]									X
Research breast tissue collection[l]	X		X				X		
DCE-MRI[n]	X		X						
Research blood collection[o]		X			X	X		X	

A: Eribulin mesylate – 1.4mg/m^2 IV day 1, 8 every 21 days x 4 cycles. One cycle = 21 days.

B: AC – doxorubicin 60mg/m^2 IV and cyclophosphamide 600mg/m^2 IV day 1 every 14 days x 4 cycles. One cycle = 14 days. Begin AC 2 weeks (14 days +/2 days) after cycle 4, day 8 of eribulin.

a: Physical examination performed on Day 1 of every cycle (+/- 2 days). Physical examination must be performed by a medically qualified individual such as a licensed physician, physician's assistant, or advanced registered nurse practitioner. b. Vital signs should include: BP, HR, respiratory rate, body temperature and weight. Height performed at baseline only.

c: If screening labs done ≤ 7 days of C1D1, they do not need to be repeated

d: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.

e: Magnesium only required at C5D1 (prior to first dose of eribulin).

f: Serum or urine pregnancy test (women of childbearing potential). g: ECHO is preferred but MUGA will be accepted.

h: Breast imaging must be done 1-2 weeks after completing preoperative treatment, prior to surgery.

i: Breast imaging per standard practice recommendations. j: Surgery to occur 3 to 5 weeks following completion of AC.

k: Radiation therapy to begin approximately 3 to 6 weeks following surgery. Endocrine therapy (for ER and/or PR positive disease) per institutional standards may begin concurrent with or after completion of radiation therapy per physician preference.

l: Breast biopsy performed at baseline and prior to cycle 1, day 8. Research biopsies can be done the same day as treatment. Baseline biopsy can be done any time prior to initiating treatment, cycle 1, day 1. Cycle 1 day 8 biopsies can be done within 1 day prior to therapy. Tissue is also to be collected at the time of surgery. See section 9.2 for tissue collection guidelines.

m: Every 3 months (+/- 1 month) x 1 year, then every 6 months (+/- 1 month) x 4 years, then annually. Patients can be evaluated at any time as deemed clinically necessary by the investigator.

n: DCE-MRI performed in 5 patients. Imaging performed at baseline and prior to cycle 1, day 8. Baseline imaging can be performed any time prior to initiating treatment (cycle 1, day 1) but imaging must be performed after the research breast biopsy at baseline and the second DCE-MRI must be performed prior to the cycle 1, day 8 research biopsy. DCE-MRI of the affected breast is repeated on the same scanner (Siemens 3T scanner). There must be between 5-8 days between both DCE-MRI scans.

o: Research blood samples collected at screening OR C1D1, C5D1, pre-surgical evaluation and post-surgical evaluation. See Section 9.1 for details.

11. MEASUREMENT OF EFFECT

11.1 Clinical Evaluations

For the purposes of this study, participants should be reevaluated during preoperative therapy every 1 cycle by physical examination. Photographs of the affected breast may be obtained. If extensive nodal involvement is present, patients should be evaluated with scans prior to cycle 5 day 1 (first dose of AC for Cohort A or first dose of eribulin for Cohort B) and prior to surgery.

Tumor response will be evaluated based upon resolution of edema, erythema and any density palpable within the effected breast. Breast imaging and clinical exam will determine final criteria for surgical respectability.

In the case of skin changes, documentation by color photography is recommended.

11.2 Pathological Response Parameters

Pathologic Complete Response (pCR)

Complete pathologic disease response (pCR) is defined as absence of invasive carcinoma within the breast and axillary lymph nodes following preoperative therapy. Participants whose disease is not surgically resectable following preoperative treatment are considered as not having pCR.

Residual cancer burden (RCB)

Residual cancer burden (RCB) after preoperative therapy will be determined, as defined by Symmans et al.[35]

11.3 Disease-Free Survival, Time to Treatment Failure, Overall Survival

- Overall Survival: Overall Survival (OS) will be defined two ways: among patients who undergo surgery, as time from surgery until death from any cause; and among all patients, as the time from treatment initiation until death from any cause. Censoring will use the date last known alive.

Disease-Free Survival: Disease-Free Survival (DFS) will be defined among patients who undergo surgery, as time from surgery until occurrence of one of the events below.

- Ipsilateral local-regional invasive breast cancer recurrence (i.e., an invasive breast cancer in the axilla, regional lymph nodes, chest wall and / or skin of the ipsilateral breast, or skin of the contralateral breast)

- Distant recurrence (i.e., evidence of breast cancer in any anatomic site other than local-regional disease described above) that has either been histologically confirmed or clinically diagnosed as recurrent invasive breast cancer.

- Contralateral invasive breast cancer.

- Death attributable to any cause including breast cancer, non-breast cancer, or unknown cause (but cause of death should be specified if at all possible).

- Second primary cancers other than breast.

Time to Treatment Failure: Time to treatment failure (TTF) will be defined among all patients from time of treatment initiation until occurrence of one of the events below or occurrence of progressive disease during preoperative therapy or treatment of disease that is not surgically resectable.

- Ipsilateral local-regional invasive breast cancer recurrence (i.e., an invasive breast cancer in the axilla, regional lymph nodes, chest wall and / or skin of the ipsilateral breast, or skin of the contralateral breast) .

- Distant recurrence (i.e., evidence of breast cancer in any anatomic site other than local-regional disease described above) that has either been histologically confirmed or clinically diagnosed as recurrent invasive breast cancer.

- Contralateral invasive breast cancer.

- Death attributable to any cause including breast cancer, non-breast cancer, or unknown cause (but cause of death should be specified if at all possible).

- Second primary cancers other than breast.

For DFS and TTF, patients without an event will be censored at the date of last disease assessment.

12. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

12.1 Data Reporting

Method

The ODQ will collect, manage, and perform quality checks on the data for this study.

Responsibility for Data Submission

Investigative sites within DF/HCC or DF/PCC are responsible for submitting data and/or data forms to the ODQ according to the schedule set by the ODQ.

12.2 Data Safety Monitoring

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this study. The committee is composed of clinical specialists with experience in oncology and who have no direct relationship with the study. Information that raises any questions about participant safety will be addressed with the Overall PI and study team.

The DSMC will review each protocol up to four times a year or more often if required to review toxicity and accrual data. Information to be provided to the committee may include: up-to-date participant accrual; current dose level information; DLT information; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring with 30 days of intervention for Phase I or II protocols; for gene therapy protocols, summary of all deaths while being treated and during active follow-up; any response information; audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

12.3 Collaborative Research and Future Use of Data and Biospecimens

Tissue, blood, bodily fluids, and other materials derived from these will be collected in this study to analyze genes, DNA, RNA, proteins and cells for the study's correlative endpoints and potential future research, utilizing new types of biomarker testing as it becomes available.

These samples and any data generated as a part of these clinical trials may be used for future research studies and may be provided to collaborating investigators both within and outside of the DF/HCC for either correlative endpoints or secondary use. Samples and data may be shared with outside non-profit academic investigators, as well as with for-profit pharmaceutical investigators or commercial entities, with whom we collaborate. When samples or data are sent to collaborators and when any research is performed on them, all information will be identified with a code, and will not contain any PHI, such as name, birthday, or MRNs.

In order to allow the greatest amount of research to be performed on the specimens and information generated as a part of this trial, researchers in this study may share results of genetic sequencing with other scientists. De-identified specimen or genetic data may be placed into one of more publicly-accessible scientific databases, such as the National Institutes of Health's Database

for Genotypes and Phenotypes (dbGaP). The results from the correlative research on this study will be shared with these public databases. Through such databases, researchers from around the world will have access to de-identified samples or data for future research. More detailed information, beyond the public database, may only be accessed by scientists at other research centers who have received special permission to review de-identified data.

13. STATISTICAL CONSIDERATIONS

The primary objective of this phase II trial is to determine pathologic complete response (pCR) rate after preoperative therapy with either 4 cycles of eribulin mesylate (E) followed by 4 cycles of doxorubicin/cyclophosphamide (AC) or after 4 cycles of AC followed by 4 cycles of eribulin in HER2 negative inflammatory breast cancer, as well as to assess the residual cancer burden (RCB) after preoperative therapy.

In the original design participants in Cohort A were to be enrolled using a single-arm, two-stage design. Cohort A did not meet the criteria to proceed to the second stage, with 1/16 patients achieving pCRs (0.0625; 90% CI 0.0032 to 0.2638). Cohort B are enrolled using a single-arm, two-stage design, with revised design criteria.

The original study (Cohort A) was designed based upon published literature suggesting a 25% pCR rate following anthracycline/taxane preoperative therapy in ER- IBC.[8] . More recent literature in IBC specifically and generally across all early breast cancer, indicate pCR rates are lower in ER+ than ER- disease (especially ER+/HER2-), which were the majority of patients enrolled in Cohort A. We have subsequently estimated that the pCR rate for ER+/HER2- IBC is significantly lower than 25%, approximately 2% based upon an analysis of DFCI IBC registry data (DF/HCC 11-035). For this reason, we modified the null and alternative hypotheses for Cohort B, as well as the α error, and plan enrollment of 16 patients, as was done for the first stage of Cohort A.

In both cohorts, following surgery, radiation therapy and endocrine therapy are given according to standard of care. Participants will be followed post-therapy for recurrence and survival. Efficacy of protocol therapy, defined as disease-free survival (DFS), time to treatment failure (TTF), and overall survival (OS), will also be assessed.

For correlative studies, research biopsies will be obtained prior to start of preoperative therapy, and prior to day 8 of cycle 1 of preoperative therapy. A sample of any residual disease within the breast at time of surgery will be obtained.

13.1 Study Design/Endpoints

13.1.1 **Primary Endpoint.**

The primary endpoint is pCR, as defined in Section 11.2.

13.1.2 **Design**

The study (Cohort A) was planned using Simon minimax two-stage design. We propose that if the proportion of participants experiencing pCR is ≤ 0.10 then the preoperative EAC regimen is considered minimally effective, versus an alternative hypothesis that the EAC regimen is worthy of further study if proportion pCR ≥ 0.30. After testing the regimen on 16 participants in the first stage, the study will be terminated if ≤ 1 have pCR, and will proceed if ≥ 2 of 16 have pCR. If the study goes on to the second stage, the regimen is considered worthy of further study if ≥5 of 25 participants in total have pCR.

If the preoperative EAC regimen is actually not effective, there is an α=0.095 probability of concluding that it is (target α=0.10); if the EAC regimen is actually effective, there is an 0.097 probability of concluding that it is not (target β=0.10).

For Cohort B, the design will use a two-stage design. We propose that if the proportion of participants experiencing pCR is ≤ 0.02 then the preoperative ACE regimen is considered minimally effective, versus an alternative hypothesis that the ACE regimen is worthy of further study if proportion pCR ≥ 0.23. After testing the regimen on 12 participants, the if no patient has pCR then enrollment will cease; whereas if ≥1 patient has pCR then enrollment will proceed to 16 patients. The regimen is considered promising if ≥ 2 of 16 have pCR. If the regimen is actually not worthy of further study, there is an α=0.038 probability of concluding that it is (target α=0.05); if the regimen is actually effective, there is an 0.098 probability of concluding it is (target β=0.10). If 2 of 16 patients have pCR then the two-sided 90% CI unadjusted for the two-stage design is (0.0226 to 0.3438).

13.1.3 **Primary Analysis**

The number and proportion of participants achieving pCR following preoperative treatment will be summarized with 2-sided confidence interval (CI), separately for each cohort. For Cohort A, both 80% and 90% CI will be reported; for Cohort B, 90% CI will be reported. The pCR rates will also be summarized according to hormone receptor status (ER and/or PR positive, or triple-negative).

13.2 Sample Size, Accrual Rate and Study Duration

If the trial continues to the second stage, then 25 patients will be enrolled (Cohort A); otherwise 16 patients will be enrolled in the first stage. In Cohort B, 16 patients will be enrolled. If a patient does not initiate protocol therapy, she will be replaced. Accrual is expected to be 10-12 patients per year for 2 years. Given the historic data of median OS among IBC patients with ER positive (3.2 years) or triple negative (2.0 years), the study duration to assess OS is anticipated to last for at least 5 years. [36, 37]

Accrual Targets					
Ethnic Category	**Sex/Gender**				
	Females		**Males**		**Total**
Hispanic or Latino	1	+		=	
Not Hispanic or Latino	24	+		=	
Ethnic Category: Total of all subjects	25	+	0	=	25
Racial Category					
American Indian or Alaskan Native		+		=	
Asian	1	+		=	
Black or African American	1	+		=	
Native Hawaiian or other Pacific Islander	0	+		=	
White	23	+		=	
Racial Category: Total of all subjects	25	+	0	=	25

13.3 Stratification Factors

N/A

13.4 Interim Monitoring Plan

See Section 12.2

13.5 Analysis of Primary Endpoints

See Section 13.1

13.6 Analysis of Secondary Endpoints

Therapeutic Efficacy Objectives

Time-to-event distributions for DFS, TTF and OS will be summarized using the method of Kaplan-Meier and two-sided 90% CI for the medians will be summarized (endpoints defined in Section 11.3), separately by cohort. Exploratory analyses will summarize postsurgery DFS and post-surgery OS distributions according to pCR and/or according to

RCB and estimate hazards ratios with 90% CI using Cox proportional hazards models, for the two cohorts combined.

Residual Cancer Burden following preoperative treatment is defined according to Symmans et al[35]. The number and proportion of participants achieving RCB-0, I, II, III will be summarized with two-sided 90% exact binomial CIs.

Correlative Objectives

Assess the changes in gene expression following administration of 1 cycle of eribulin, as seen in preclinical models:

The paired expression levels of each gene at the two time points will be summarized graphically and descriptively. Wilcoxon signed rank test will be used to determine if there are any significant changes in the expression level of each gene between Biopsy 1 and Biopsy 2, and secondarily between and Biopsy 1 and Mastectomy among the subgroup who do not achieve pCR (i.e., have residual invasive tumor in breast or lymph nodes at mastectomy). If 25 patients are enrolled, and assuming 80% (n=20) have adequate RNA and expression data in paired biopsy samples, there is 80% power to detect a 0.6 SD change using a signed rank test (two-sided =0.10).

Exploratory analysis will be performed to determine if there is any correlation between the changes in imaging (k^{trans}, v_e, v_p and iAUC) and genomic changes determined on core biopsies of the breast sampled at the same time points.

Assess the association of changes in gene expression with pCR and RCB at mastectomy: Exploratory analyses will summarize gene expression changes separately according to pCR and RCB status. The analyses will be descriptive.

The association of correlative biomarkers and clinical outcomes will be exploratory and hypothesis-generating and will not adjust for multiple comparisons in any statistical inferences.

In additional exploratory analyses, the changes will be dichotomized at the median, and post-surgery DFS and OS summarized according to degree of change using KM method.

13.7 Reporting and Exclusions

Evaluation of Toxicity

The frequencies of adverse events (AE) while on study treatments will be summarized and tabulated according to treatment phase (preoperative vs. post-surgical) and at any time.

Enrolled patients who received at least one dose of study treatment will be included in the analyses.

Evaluation of the Primary Efficacy Endpoint

All participants who initiate the preoperative treatment phase will be considered as evaluable for the preoperative treatment endpoints of pCR and RCB. Patients who do not initiate preoperative treatment would also be excluded from analyses of time-to-event endpoints.

14. PUBLICATION PLAN

The data will be collected and analyzed by Dr. Filipa Lynce, Dr. Meredith Regan, ScD from the Department of Biostatistics and Computational Biology, and key co-investigators. The results will be shared with [redacted] in advance of publication. It is anticipated that results will be made public within 12 months of the end of data collection. Initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors. The primary endpoint of the study will not be reported until the study is closed to accrual and there is adequate follow-up time to estimate the response rate. A report will also be published in a peer-reviewed journal. A full report of the outcomes will be made public no later than three (3) years after the end of data collection.

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APPENDIX A: PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		Karnofsky Performance Scale	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (*e.g.*, light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

APPENDIX B: PROCEDURES FOR RESEARCH CORE BIOPSY OF BREAST

Site: DFCI

Biopsy Tissue Collection Protocol: I.
Supplies:

- Tissue-Tek Cryomold
- Standard size 25x20x5mm (#4557)
- Fisher Scientific Cat# NC9511236
    - OR
- Intermediate size 15x15x5mm (#4566)
- Fisher Scientific Cat# NC9542860
- Tissue-Tek O.C.T. Compound, 4oz (#4583): Fisher Scientific Cat# NC9638938
- Small specimen bag: Fisher Cat# 01-002-37 (or equivalent)
- Forceps: Fisher Cat# NC9832137 (or equivalent)
- Dry Ice and Cooler
- Cryoware Pen: Fisher Cat# 13-382-88
- Protocol® 10% Zinc Formalin Cat# 032-065

II. Procedure:

- Prepare cryomolds ahead of time: Squeeze small amount of OCT into cyromolds* – enough for there to be a thin layer covering the bottom let settle on a flat surface for a minute or two. It is *crucial* for future sectioning that these core biopsies are placed on a very flat OCT surface, so the best way to make sure these mold are flat is to *freeze the bottom layer on a -80 freezer shelf*.
- Bring all supplies needed to the biopsy as the tissue needs to be frozen or fixed in formalin immediately.
- You should ideally collect between 5 and 8 separate cores.Priority for core collection:
    1. Frozen in OCT
    2. Frozen in OCT
    3. Formalin (for FFPE)
    4. Formalin (for FFPE)
    5. And any additional cores: Frozen in OCT
    - <u>For frozen samples</u>: As soon as the specimen is ready, use disposable forceps to gently place only 1 core into each cryomold. Gently pick up the tissue with the forceps from one end of the core biopsy, being careful not to crush the tissue and immediately lay out the fresh biopsy tissue onto the center of the mold. Be sure to lay the tissue as flat to the mold, and as straight as possible. Keep molds on dry ice at all times.
    - Once the specimen is in the cryomold, cover with more OCT making sure the tissue is entirely submerged.
    - Immediately place cryomold with OCT and tissue onto dry-ice making sure the cryomold is level and will not tip over.
    - The OCT will freeze into a solid white block within 5-10 minutes
    - Once the blocks have completely frozen they can be put into a specimen bag and sealed. More than one block can be put into a bag.
    - The bag should be labeled with:
    - Patient name
    - DFCI Study #:
    - DFCI MRN #
    - Date of biopsy
    - Time Point (Biopsy #1, Biopsy #2, or Surgery)
    - Number of blocks in the bag

- For formalin fixed samples: As soon as the specimen is ready, use disposable forceps to gently place only 1 core into each formalin jar.
- The formalin containers should be labeled as bags above.
- All frozen research tissue should then be brought to the DF/HCC Core Blood and Tissue Bank on the day of collection. Samples in formalin should be brought to the BWH Specialized Histopathology Core. **Cryomolds should be kept on dry ice at all times.**

*If time permits, the cryomolds can be placed onto the dry-ice once a thin layer of OCT has been put it but before the tissue is put in. Once this is frozen or begins to freeze, the tissue can be placed on top of the now frozen OCT and then covered with more liquid OCT and then placed back onto the dry ice to freeze completely.

APPENDIX C: MASTECTOMY TISSUE COLLECTION PROTOCOL

I. Procedure:

- Keep tissue on dry ice in insulated container as it is transported to the Institutional Tissue Bank.
- Place tumor into sterile petri dish in a cryostat at -20°C. Using a sterile scalpel blade, cut two to four adjacent slices from the tumor which are representative of the tumor and which are grossly free of stroma/fat and necrosis. There is no limitation on size of tissue obtained so long as the residual tumor within the mastectomy specimen is $\geq$ 1 cm. One to two sections are placed immediately into formalin until it is collected by the study coordinator to be processed into paraffin embedded blocks.
- The other one to two pieces of tumor are placed on a chuck, layered with OCT, and allowed to freeze. One block is faced off and multiple 5 μm thick sections are placed on a charged and labeled glass slide. The slide is stained with hemotoxylin and eosin (H+E), coversliped, and then evaluated for tumor percentage (T), stroma/fat/blood percentages (S), and necrosis percentage (N). The tumor percentage and stroma/fat/blood percentage should be based on area occupied by nuclei and not by that of cytoplasm. These results are written on the slide, recorded in the database, and the slides are stored in the Tissue Bank.

*If time permits, the cryomolds can be placed onto the dry-ice once a thin layer of OCT has been put it but before the tissue is put in. Once this is frozen or begins to freeze, the tissue can be placed on top of the now frozen OCT and then covered with more liquid OCT and then placed back onto the dry ice to freeze completely.

APPENDIX D: BIOASSAY TEMPLATES

RNA extraction from frozen tumor specimens

Frozen sections (20 micron thickness) will be cut from tissue cores embedded in OCT. One slide from each specimen will be stained according to standard protocols with Haematoxylin and Eosin. Only samples with at least 50% tumor cellularity will be included in the analysis.

Total RNA will be extracted from samples according to standard in-house protocols. RNA concentration and purity will be measured using the NanoDrop 1000 apparatus (ThermoScientific). RNA will then be stored at -80C until required for use.

Reverse transcription

RNA will be reverse transcribed using the iScript Reverse Transcription Supermix (Bio-Rad) according to manufacturer's instructions. A total of between 200ng and 1000ng of RNA will be reverse transcribed, depending upon the amount of total RNA available from each sample. cDNA will be stored at -20C until utilized.

Primer Design

A single primer pair will be designed for RT-qPCR to each of the genes described in the protocol. Primers will be designed using the Primer-BLAST online tool (National Centre for Biotechnology Information) with attention paid to GC content (50-60%), melting temperature (50-65 degrees C), avoidance of secondary structure targeting, and minimization of 3'- and 5'-primer complementarity to avoid primer-dimer formation. Primers will be ordered through the Dana-Farber Cancer Institute's Molecular Biology Core Facility.

RT-qPCR assays

RT-qPCR will be performed in 96-well optical plates using an QT-qPCR instrument held in the Department of Cancer Biology, Dana-Farber Cancer Institute (Applied Biosystems). Each RT-qPCR assay will be performed in triplicate, in addition to triplicate assays for two housekeeping genes.

RT-qPCR will be set up in 20uL reactions per well as follows:

1. SYBR qPCR Mastermix (BioRad): 10uL
2. 3'-primer (5 pmol/uL): 0.3uL
3. 5'-primer (5 pmol/uL): 0.3uL
4. Nuclease free water: 7.4uL
5. cDNA template: 2uL

the RT-qPCR cycling conditions will be as follows:

1. 50°C 2 min, 1 cycle
2. 95°C 10 min, 1 cycle
3. 95 °C 15 s -> 60 °C 30 s -> 72 °C 30 s, 40 cycles
4. 72°C 10 min, 1 cycle

DANA-FARBER CANCER INSTITUTE
Nursing Protocol Education Sheet

Protocol Number:	**15-292**
Protocol Name:	A Phase 2 Study of Eribulin Followed by Doxorubicin and Cyclophosphamide as Preoperative Therapy for HER2-negative Inflammatory Breast Cancer
DFCI Site PI:	Filipa Lynce MD
DFCI Research Nurse:	Margaret Haldoupis, Elizabeth Kasparian, Kathleen Roche, , Janet LaPointe, Victoria Brock; Christine Wong, Jaclyn Colucci

Page the DFCI research nurse or DFCI site PI if there are any questions/concerns about the protocol.
Please also refer to ONC 15: Oncology Nursing Protocol Education Policy
***** Remember to check the ALERT PAGE*****

SPECIAL NURSING CONSIDERATIONS UNIQUE TO THIS PROTOCOL

Study Agent	**Eribulin** is a synthetic derivative of the natural product halichondrin B, which is isolated from a marine sponge. Halichondrin B exhibits anti-cancer activity through a microtubule-destabilizing anti-mitotic mechanism of action. Eribulin exerts its effects by binding to microtubules which causes interference in the normal cell cycle. This interference leads to cell death via apoptosis. **Doxorubicin** is a cytotoxic Anthracycline antibiotic. **Cyclophosphamide** is a nitrogen mustard alkylating agent. A cycle is defined as 21 days, cycles 1-4 of Eribulin, then every 14 days during cycles 5-8 of AC (Section 5.1)
Dose Calc.	• Eribulin is dosed in mg/m2-**Section 5.1.** Doxorubicin is dosed in mg/m2 • Cyclophosphamide is dosed in mg/m2 For All 3 Study Drugs: Body weight will be recorded on each treatment day and dosing BSA will be calculated per Institutional Guidelines. (Section 5.3).
Study Drug Administration	Agent *Administration* Guidelines are found in Section 5.3 • **Eribulin** IV administered over approximately 2 to 5 minutes on Days 1 and 8 of every 21 day cycle for a total of 4 cycles. (Section 5.3) • **Doxorubicin and Cyclophosphamide** are administered concurrently (AC) beginning 2 weeks after the last dose of Eribulin is given (cycle 4, day 8). • **Doxorubicin** 60 mg/m2 will be administered on an outpatient basis via I.V. push over approximately 3 to 5 minutes on Day 1 of each 14 day cycle, for a total of 4 cycles. • **Doxorubicin** is a vesicant, and is administered per Institutional Standard. • **Doxorubicin** is administered **immediately prior to** the administration of Cyclophosphamide. • **Cyclophosphamide** 600 mg/m2 will be administered on an outpatient basis via I.V. infusion over approximately 30 minutes on Day 1 of each 14 day cycle, for a total of 4 cycles. • **Cyclophosphamide** is diluted in 250-500 ml normal saline, and its administration is per institutional standard/package insert. • **Cyclophosphamide** is administered immediately following the administration of doxorubicin. • If AC is delayed due to toxicity, drug administration every 3 weeks on a 21 day cycle may be given with the approval of the Principal Investigator. (Section 6.2) • Endocrine Therapy should be administered in Participants with EER/PR positive disease as defined by Institutional guidelines.
Dose Mods & Toxicity	*Criteria to Treat, Dose Modifications/Dosing Delay for Toxicity* are outlined in Section 5.6 • This protocol uses NCI CTCAE criteria, version 4.0 • Criteria to Treat is found in Section 5.2 • Expected toxicities are outlined in Section 7.1 • Dose modifications and delays for all drugs are outlined in Section **6.1** tables 1 and 2 for Eribulin. • See Section **6.2,** and tables 3 and 4 for Doxorubicin and Cyclophosphamide • Maximum amount of dose reductions for Eribulin is 2. (Section 6.1)If Eribulin is held for greater than 2 weeks for toxicity, it should be discontinued. (Section 6.1) • Management of toxicities related to Eribulin are listed in Table 2.

	• No more than 1 dose-reduction of AC chemotherapy should be made for toxicity. (Section 6.2) If AC is held for >2 weeks for toxicity, Participants should be discontinued. (Section 6.1) Management of toxicities related to AC are listed in Table 4.

Con Meds	*Concomitant Therapy* Guidelines are in Section 5.4 • Permitted meds are in Section 5.4 • Platelet growth factors are specifically prohibited. (Section 5.4) • No Erythropoietin allowed while on study. (Section 5.4) • GCSF is allowed during Doxorubicin / Cyclophosphamide cycles 5-8
Req Data	***Study Calendar and Assessment Required data* are outlined in Section 10.0**
Tips	**All study drugs require documentation of exact administration time.**

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