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DEPARTMENT OF MEDICAL ONCOLOGY AND THERAPEUTIC RESERCH

TITLE: **PHASE I/IB TRIAL OF ERIBULIN AND EVEROLIMUS IN PATIENTS WITH TRIPLE NEGATIVE METASTATIC BREAST CANCER**

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STUDY SPONSOR AND MONITOR:

Study Synopsis and Design

Study Rationale

Patients with metastatic breast cancer (MBC) have a median overall survival (OS) of approximately 27 months. While OS has improved due to the introduction of new therapeutic agents, drug resistance is an ongoing challenge in the treatment of patients with MBC. Most patients with MBC become resistant to standard chemotherapeutic agents such as anthracyclines and taxanes or platinums; and while the development of hormonal therapies and anti-HER2 therapies have revolutionized treatment for patients with hormone receptor-positive and HER2-positive breast cancer, patients with hormone receptor-negative/HER2-negative cancer (triple negative breast cancer, TNBC) are in need of more efficacious therapies.

Mutations in the PI3K pathway are the second most common mutations in breast cancer, and are the most common activating mutations, found in as many as 26%-45% of breast cancers. Triple negative breast cancers demonstrate higher levels of AKT activation, with consequent activation of the mammalian target of rapamycin (mTOR) pathway downstream of AKT. Everolimus inhibits cytokine and growth-factor-dependent cell proliferation by inhibiting the mTOR pathway. Everolimus has shown significant anti-tumor activity, but since the PI3K-mTOR pathway is downstream of numerous other effectors known to play a role in cancer cell proliferation and/or survival, there is an increasing focus on the drug's possibilities when combined with other anti-tumor therapies. Increased efficacy has been observed when everolimus is combined with many other drugs. Eribulin mesylate is a synthetic analog of halichondrin B, a natural product isolated from the marine sponge Halichondria okadai. It acts as a cancer therapeutic via a tubulin-based anti-mitotic mechanism, which leads to G2/M cell cycle arrest, disruption of mitotic spindles, and ultimately apoptotic cell death. It inhibits cell growth in a wide range of cancer cell lines and in patients with advanced solid tumors and MBC.

Based on our preclinical findings, we hypothesize that the combination of everolimus and eribulin will have a synergistic effect and will increase clinical activity in patients with metastatic TNBC. Previous studies of each of these drugs in MBC show acceptable toxicity, and we hypothesize that this drug combination could offer a novel approach to treat patients with metastatic TNBC who are resistant to anthracyclines and taxanes or platinums.

Objectives

The overall objective of this study is to conduct a phase I/Ib trial of everolimus plus eribulin in metastatic TNBC patients who progressed after anthracyclines and/or taxanes or platinums. The trial will consist of a Phase I trial and with an extension to Phase Ib.

Primary Objective for Phase I Study

The primary objective of the Phase I portion of the study is to determine the safety and tolerability of everolimus and eribulin, and determine the recommended PhaseII dose (RP2D) of

the drug combination in patients with resistant metastatic TNBC.

Primary Objective for Phase Ib Study

The primary objective of the Phase Ib portion of the study is to evaluate the event-free survival (EFS) rate for patients with resistant metastatic TNBC at the RP2D of everolimus and eribulin to determine if the drug combination is worthy of further study.

Secondary Objectives for Phase Ib Study

The secondary objectives of the Phase Ib portion of the study are to determine response rate, OS, toxicity, and pharmacokinetics (PK) for everolimus and eribulin in patients with resistant metastatic TNBC.

A further secondary objective is to collect blood, skin punch biopsies, and tumor biopsies before and after treatment from all patients, and perform proteomic analysis to determine the level of inhibition of the PI3K pathway in tumor cells versus non-therapeutic targets.

Study Design

This is a Phase I/Ib trial of everolimus plus eribulin in metastatic TNBC patients who are resistant to anthracyclines and taxanes or platinums.

Phase I Study

The Phase I portion of the trial will use the toxicity equivalence range (TEQR) design. Based on this design, the target equivalence range for dose-limiting toxicities (DLTs) is 0.20-0.35. Toxicity levels of $\geq$0.51 will be considered too toxic, and doses which achieve this level will be stopped. Patients will enter the protocol in cohorts of three. This phase will be considered complete when 12 patients are studied at a single dose level with a toxicity level <0.51. The RP2D will be the dose closest to the target of 0.25 below 0.51 based on isotonic regression. Based on these requirements, the TEQR design will use a minimum of 6 patients and a maximum of 24 patients (median of 18) to determine the RP2D.

The PMT team for protocol 14036 reviewed the data: dose level 2 was closed after 2 of 3 evaluable participants experienced DLTs at an observed rate of 67%; for dose level 1, we saw 4 DLTs in 8 evaluable participants giving an observed DLT rate of 50% on this dose. Our equivalence range for DLT rate for this trial is 20-35%, our observed rate of 50% is above 35% so we plan to de-escalate. Two participants experienced grade 4 neutrophil count decrease and 2 experienced mucositis (1 grade 3 DLT, 1 participant received 71% of Everolimus due to grade 2 which is a DLT). Thus, the team decided to amend the protocol to allow for two new dose levels at a lower dose of Eribulin, and start the next cohort of patients at dose level B1.

Dosing Schedule: cycle length is 21 days

Dose level A	Everolimus (oral)	Eribulin (intravenous)
A-1	2.5mg daily (day 1 to 21)	1.4mg/m^2 on days 1, 8
A1	5mg daily (day 1 to 21)	1.4mg/m^2 on days 1,8
A2	7.5mg daily (day 1 to 21)	1.4mg/m^2 on days 1,8
A3	10mg daily (day 1 to 21)	1.4mg/m^2 on days 1, 8

Dose level B	Everolimus (oral)	Eribulin (intravenous)
B1	5mg daily (day 1 to 21)	1.1mg/m^2 on days 1,8
B-1	2.5mg daily (day 1 to 21)	1.1mg/m^2 on days 1,8

Phase Ib Study

In the Phase Ib portion of the trial, a Simon's Optimal two-stage design will be used to determine if the combination of everolimus and eribulin results in a greater rate of patients who are event-free at 4 months than with eribulin alone. Based on data from the EMBRACE trial, we set the 4-month EFS rate for eribulin at 45%. We want to determine if the drug combination has a 4-month EFS rate of ≥70% (25% improvement). Setting both the type I and type II errors to 0.1 after 12 patients (from the RP2D at the end of the phase I trial), if ≤5 patients are event-free at 4 months, the study will stop accrual. If ≥6 patients are event-free at 4 months we will enroll 15 more patients for a total of 27 patients at the RP2D. If ≥16 patients out of the 27 are event-free at 4 months we will consider the combination worthy of further study.

The rationale for calling this a Phase Ib trial is that this trial is a large combination Phase I trial that will indicate if there is some activity of the drug combination, but it will not provide the activity information that a randomized Phase II trial would provide. Nonetheless, it still will provide information about whether the drug combination should be studied further.

Study Endpoints

Primary Endpoint

The primary endpoints of the study are:

- Phase I: DLT.
- Phase IB: EFS at 4 months.

Secondary Endpoints

The secondary endpoints are:

- Phase Ib: Response rate, PFS, OS, toxicity profile and PK parameters

Sample Size and Accrual Rate

The expected combined sample size for the Phase I/Ib trial will be 33. We anticipate 18 study participants for Phase I portion, and 15 additional at the RP2D to finish out the Phase II portion of the trial (minimum sample size=6 and maximum =45 (Phase I: 24 + Phase II: 15 + 6 for unevaluable/ineligible patients over the whole trial)).

At the time of adding schedule B, 14 participants had been treated of which 12 are evaluable for dose escalation. With the addition of 2 more doses we expect to treat 12 to 15 more patients for a total of ~29 on the phase I portion of the trial, thus we expect the trial sample size to stay within the above planned limit of 45.

Our accrual is just over 1 participant a month so the phase I portion should be completed after 2 years from start of the trial. We will need to follow the last patient for 4 months to get EFS at 4 months on the 12 patients studied at the RP2D, and expect to do the initial assessment of activity at approximately 2 1/2 years. If 6 or more of the patients studied at the RP2D are event free we will add on 15 additional patients. Based on the same accrual rate we should complete the accrual of the Phase Ib portion within approximately 12 months or at 42 months after start of the study.

Inclusion Criteria Patients with histologically confirmed stage IV TNBC (patients who had metastatic disease within 6 months of lumpectomy or mastectomy for treatment of TNBC may be excused from repeat biopsy) with measurable disease;

Be willing to provide tissue from a newly obtained core or excisional biopsy of a tumor lesion. Newly – obtained is defined as a specimen obtained up to 6 weeks (42 days) prior to initiation of treatment on day 1. Subjects for whom newly-obtained samples cannot be provided (e.g. inaccessible or subject safety concern) may submit an archived specimen only upon agreement from the study PI.

Chemotherapy for metastatic disease (patients with 0-3 lines of chemotherapy for MBC)

Prior radiation therapy allowed;

Patients who previously received anthracyclines and/or taxanes or platinums ;

age ≥18 years;

Life expectancy ≥3 months;

Eastern Cooperative Oncology Group performance score (ECOG PS) 0- 2;

Adequate bone marrow, liver and renal function;

Be willing to use dexamethasone mouth wash as directed.

Exclusion Criteria

Patients may not be receiving any other investigational agents; patients received chemotherapy and/radiation therapy within 2 weeks from study entry; patients with symptomatic brain metastases; uncontrolled current illness including, but not limited to, ongoing or active infection (>Grade 2), symptomatic congestive heart failure, unstable angina pectoris, myocardial infarction within the past 6 months, cardiac ventricular arrhythmias requiring anti-arrhythmic therapy, or psychiatric illness/social situations that would limit compliance with study requirements; pregnant women; prior eribulin use; patients with known history of HIV; chronic hepatitis B; or chronic hepatitis C ; Concomitant use with strong or moderate CPY3A4/PgP inhibitors and CPY3A4/PgP inducers is not permitted; patient who is noncompliant with oral medication and mouth wash regimen.

Clinical Observations and Tests to be Performed

Pre-study assessments should be performed within 28 days prior to initiating therapy.

We will assess tumor response using response evaluation criteria in solid tumors (RECIST) Version 1.1 criteria based on radiologic imaging every 2 cycles of treatment (6 weeks).

Toxicity will be evaluated based on the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) v4.0.

Patients enrolled in the expanded phase Ib cohort at the RP2D will also be asked to undergo serial blood sampling to evaluate the PK of everolimus and eribulin. Although it is unlikely that these two agents will interact negatively, both drugs are substrates for the membrane transporter P-glycoprotein. Therefore, PK studies will be performed in the expanded cohort treated at the RP2D to evaluate the PK of the drug combination.

For correlative studies, blood will be collected from patients who consented at the prior time points: prior to the first dose of everolimus and eribulin, at 4 and 8 hours after the first dose of everolimus and eribulin on day 1, and at two post-dose time points on days 4 and 8 of the first cycle for patients enrolled on the expanded cohort of RP2D.

Two sets of optional punch biopsies (two biopsies per set for a total of four biopsies) will be performed on patients who consented as follows:

- First set
    - Biopsy 1 (3mm diameter) will be performed one week prior to any dosing.
    - Biopsy 2 (3mm diameter) will be performed on the same site as Biopsy 1 on the day of the first dose of eribulin and everolimus; cycle 1 (prior to any dosing).

- Second set

- Biopsy 3 (3mm diameter) will be performed on a new site on the day of the first dose of eribulin and everolimus; cycle 1 (prior to any dosing).
- Biopsy 4 (3mm diameter) will be performed on the same site as Biopsy 3, one week later on the day that the second dose of eribulin is given (prior to dosing).

Patient will be asked to provide a fresh tumor tissue from a newly obtained core or excisional biopsy of a tumor lesion. Newly –obtained is defined as a specimen obtained up to 6 weeks (42 days) prior to initiation of treatment on day 1. Subjects for whom newly-obtained samples cannot be provided (e.g. inaccessible or subject safety concern) may submit an archived specimen only upon agreement from the study PI.

For patient who consented for optional tumor biopsy upon disease progression, a sample of tumor tissue will also be collected in patients who provided additional consent .

Serum, peripheral blood mononuclear cells (PBMC), skin biopsy tissue and tumor tissue will be processed and stored according to established laboratory protocols and stored at -80°C until proteomic analysis. Analysis will be performed by Luminex and mass spectrometry, and western blotting for pAKT (Ser473), and AKT, p4E-BP1 (ser65/Thr70) and 4E-BP1, pS6K1 (Thr389) and S6K1, and pS6 (Ser235/236) and S6.

Statistical Considerations

Tables will be created to summarize all toxicities and side effects by dose, course, organ, and severity. Rates and associated 95% confidence limits will be estimated for DLTs at the RP2D, EFS at 4 months and response. Kaplan Meier methods will be used to estimate the median and 95% confidence limits for EFS and OS. Descriptive statistics will be provided for the research participant demographics and PK parameters.

Sponsors

City of Hope is the sponsor and Novartis and Eisai will supply the drug.

SECTION **PAGE**

Abbreviations

AE	Adverse event
AKT	Protein kinase B
AUC0-τ	Area under the plasma concentration-time curve from time zero to the last measurable concentration
BHT	Butylated hydroxytoluene
CBR	Clinical benefit rate
CI	Combination indices
COH	City of Hope
CRA	Clinical research associate
CRF	Clinical report form
CTU	Clinical trials unit
DLT	Dose-limiting toxicity
ECOG PS	Eastern cooperative oncology group performance score
EDC	Electronic data capture
EFS	Event-free survival
ER	Estrogen receptor
HIPAA	Health Insurance Portability and Accountability Act
IC50	Half maximal inhibitory concentration
INR	International normalized ratio
LD	Longest diameter
MBC	Metastatic breast cancer
MTD	Maximum tolerated dose
mTOR	Mammalian target of rapamycin
NCI CTCAE	National cancer institute common terminology criteria for adverse events
OS	Overall survival
PARP	Poly ADP ribose polymerase
PBMC	Peripheral blood mononuclear cells
PD	Progressive Disease
PFS	Progression-free-survival

PI3K	Phosphatidylinositide 3-kinase
PK	Pharmacokinetics
PMT	Protocol management team
PR	Partial response
RECIST	Response evaluation criteria in solid tumors
RP2D	Recommended Phase IB dose
SAE	Serious adverse event
SD	Stable disease
TEQR	Toxicity equivalence range
TNBC	Triple negative breast cancer
TSC1/TSC2	Tuberous sclerosis complex
UP	Unanticipated problem

1.0 Background and Study Rationale

1.1 Metastatic Breast Cancer

Patients with metastatic breast cancer (MBC) have a median overall survival (OS) of approximately 27 months;[1] and median OS is even lower in previously treated patients.[2] While OS has improved due to the introduction of new therapeutic agents, drug resistance is an ongoing challenge in the treatment of patients with MBC. Most patients with MBC become resistant to standard chemotherapeutic agents such as anthracycline and/or taxanes or platinums; and while the development of hormonal therapies and anti-HER2 therapies have revolutionized treatment for patients with hormone receptor-positive and HER2-positive breast cancer,[3] patients with hormone receptor-negative/HER2-negative cancer (triple negative breast cancer, TNBC) are in need of more efficacious therapies to improve clinical outcomes.

1.1.1 Triple Negative Breast Cancer

Metastatic TNBC is an aggressive disease with a poor prognosis reflected by low rates of relapse-free survival and OS.[4] Many patients with TNBC receive anthracyclines and/or taxanes; and many develop resistant or refractory disease in the metastatic setting. Even when drug resistance is not noted, patients with TNBC have a suboptimal response to chemotherapy.[5] Since TNBC shares many features with BRCA1-associated breast cancer, which is particularly susceptible to DNA-damaging agents, platinum combination therapy, including cisplatin and carboplatin, is commonly used to treat patients with TNBC. However, studies have shown that platinum combination therapy does not result in a significant improvement in outcome when compared with other regimens. The use of poly ADP ribose polymerase (PARP) inhibitors was suggested to be a promising novel approach to the treatment of TNBC, however, the highly anticipated results of the BSI-201trial of carboplatin, gemcitabine, and olaparib in patients with TNBC showed disappointing results. New effective therapeutic approaches to treat patients with metastatic TNBC are therefore needed.

1.2 Eribulin and Everolimus

1.2.1 Eribulin mesylate (E7389)

Eribulin mesylate is a synthetic analog of halichondrin B, a natural product isolated from the marine sponge *Halichondria okadai*.[6] It acts as a cancer therapeutic via a tubulin-based anti-mitotic mechanism, which leads to G2/M cell cycle arrest, disruption of mitotic spindles, and ultimately apoptotic cell death. It inhibits cell growth in a wide range of cancer cell lines including: breast; colon; prostate; ovarian; small cell lung cancer; non-small cell lung cancer; histiocytic lymphoma; promyelocytic leukemia; pharyngeal squamous cell carcinoma; melanoma; and uterine sarcoma.[7]

Studies have assessed eribulin in advanced solid tumors defining the safety, pharmacokinetics (PK), maximum tolerated dose (MTD), and tumor response.[8] Eribulin has also been studied in patients with MBC.[9] The E7389-A001-201 study enrolled 103 MBC patients after anthracycline and taxane failure. Patients received eribulin monotherapy in two different schedules; days 1, 8, and 15 of a 28-day cycle; and days 1 and 8 of a 21-day cycle. The most common drug-related adverse events (AEs) were Grade 3-4 neutropenia (60.4%, 28-day cycle and 65.7%, 21-day

cycle) and fatigue (52.4%). The clinical benefit rate (CBR) was 17.2%. Six of the 59 (10.2%) evaluable patients on the 28-day cycle and 4/28 (14.3%) patients on the 21-day cycle had a partial response (PR).[8] The EMBRACE trial was a phase 3 open-label randomized study of eribulin monotherapy versus treatment of physician's choice in 762 MBC patients who had previously received 2-5 chemotherapy regimens for MBC (84% of patients had previous hormonal therapy). Eribulin (1.4mg/m^2) given intravenously on days 1 and 8 of a 21-day cycle showed a clinically meaningful OS improvement from 10.6 to 13.1 months (p=0.041). The CBR was 23% in the eribulin group versus 17% in the treatment of physician's choice. The most common toxicities included asthenia (54%), neutropenia (52%), peripheral neuropathy (34.6%), nausea (28.3%), and constipation (21%). Neutropenia was the most common Grade 4 AE in patients receiving eribulin (24.1%).[9]

1.2.2 Everolimus (RAD001, Afinitor)

Everolimus inhibits cytokine and growth-factor-dependent cell proliferation by inhibiting the mammalian target of rapamycin (mTOR).[10] mTOR functions as a sensor of mitogens, growth factors, energy, and nutrient levels; and it facilitates cell-cycle progression through the G1 to S-phase. The regulation of mTOR signaling is complex and involves positive regulators such as protein kinase B (AKT) that phosphorylate and inactivate negative regulators including the tuberous sclerosis complex (TSC1/TSC2). The phosphatidylinositide 3-kinase (PI3K)-mTOR pathway is frequently activated in many human cancers; and oncogenic transformation may sensitize tumor cells to mTOR inhibitors.[11, 12] Everolimus has shown significant anti-tumor activity,[13-20] but since the PI3K-mTOR pathway is downstream of numerous other effectors known to play a role in cancer cell proliferation and/or survival, there is an increasing focus on the drug's possibilities when combined with other anti-tumor therapies. Increased efficacy has been observed with all combination partners tested.[21-27]

Everolimus has been evaluated in a number of clinical trials including in patients with solid tumors and MBC.[28-30] In patients with advanced solid tumors, oral everolimus is rapidly absorbed with a median time to peak concentration of 1-2 hours post-dose. The steady-state AUC0-τ (area under the plasma concentration-time curve from time zero to the last measurable concentration) is dose-proportional over the dose range of 5-70mg in a weekly regimen and 5-10mg in a daily regimen. In a randomized, double-blind, multicenter phase III study of everolimus and exemestane versus placebo and exemestane for estrogen receptor (ER)-positive MBC, 724 patients were randomized in a 2:1 ratio to receive either 10mg daily everolimus (N=485) or matching placebo (N=239) in addition to open-label 25mg daily exemestane. The study demonstrated a statistically significant clinical benefit of the everolimus and exemestane over placebo and exemestane by a 2.4-fold increase in median progression-free-survival (PFS) (6.93 months versus 2.83 months), resulting in a 57% risk-reduction of progression or death. Objective response was observed in 9.5% of patients in the everolimus and exemestane arm versus 0.4% in the placebo and exemestane arm (p<0.0001). Furthermore, the CBR for patients receiving everolimus and exemestane was 33.4% versus 18% in patients receiving placebo and exemestane (p<0.0001.) The most common grade 3-4 AEs in the everolimus and exemestane group versus the placebo and exemestane group were stomatitis (8% versus 1%), anemia (6%

versus <1%), dyspnea (4% versus 1%), hyperglycemia (4% versus <1%), fatigue (4% versus 1%), and pneumonitis (3% versus 0%).[30]

Approximately 35,982 cancer patients have been treated with everolimus as of 31-Mar-2014:

- 19,668 patients in Novartis-sponsored clinical trials
- 2,394 patients in the individual patient supply program
- More than 13,930 patients in investigator-sponsored studies.

1.3 Rationale for the Combination of Eribulin and Everolimus in TNBC

Figure 1: MDA468 cells were treated with

Mutations in the PI3K pathway are the second most common activating mutation in breast cancer, and are the most common mutations, found in 26%-45% of breast cancers. Patients with TNBC demonstrate higher levels of AKT activation compared to other patients with breast cancers, with consequent activation of the mTOR pathway downstream of AKT. There is a well known feedback response with mTOR inhibition that seems to activate AKT. We performed preclinical evaluation of the effect of eribulin treatment on the PI3K pathway in TNBC and HER2-expressing breast cancers, both alone and in combination with everolimus.

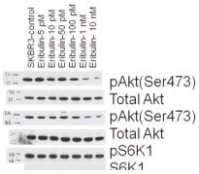


Figure 2: SKBR3 cells underwent same

We first evaluated the effect of eribulin on the growth of multiple breast cancer cell lines including TNBC cell lines. The half maximal inhibitory concentration (IC50) for eribulin ranged from 0.1nM-0.3nM in TNBC cells to ~0.06nM in the HER2-overexpressing cell line, SKBR3, to 0.5nM-0.8nM in hormone receptor-positive breast cancer cells. Importantly, eribulin clearly inhibited the phosphorylation of AKT in TNBC cells with inhibition of the Ser473 pAKT beginning between 1nM and 10nM and nearing complete inhibition at 50nM, with no effect on total AKT (**Figure 1**). In the HER2-expressing cell line, SKBR3, eribulin began to have its effect on pAKT at 10pM (**Figure 2**). This effect appeared to begin at 2-4 hours.

In TNBC cells, everolimus has effects at very low doses, but increasing doses beyond a certain concentration did not show a linear incremental improvement in cell inhibition such that in some lines an IC50 was not achieved even at 10,000nM. We then evaluated everolimus using log dilutions in combination with standard dilutions of eribulin and found synergistic growth inhibition in TNBC cell lines using the MTT assay, except for at the highest doses (**Figure 3**).

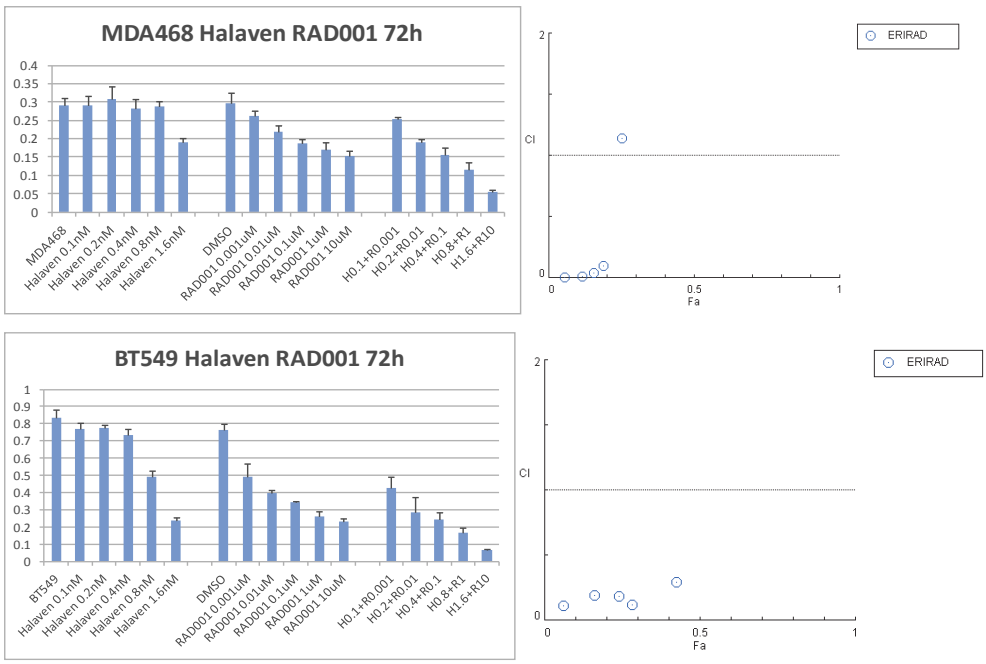
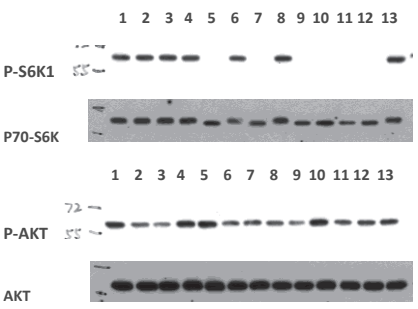


Figure 3 Two TNBC cell lines, MDA468 and BT549 are shown. Left panels: MTT assay of MDA468 and BT549 in the presence of eribulin in standard serial dilution, and everolimus in log dilution, either alone, or in combination. Right panels: combination indices (CI's) for different combinations. With the exception of eribulin at 1.6nM with everolimus at 10,000nM in MDA468 cells (H1.6+R10), all CI's are <1 (horizontal line), indicative of synergy.

We then performed western blots for pAKT and the mTOR target, pS6K1, and found that the combination of eribulin and everolimus suppressed both pAKT and pS6K1 at 24 hours; AKT was suppressed by eribulin in a dose-related fashion. A well known phenomenon associated with mTOR inhibition is the enhancement of pAKT when mTOR is inhibited, and this was seen at 24 hours with everolimus alone. However, in combination with eribulin, both the increased pAKT and pS6K1 were suppressed (**Figure 4;** lanes 7 and 9 versus lane 5).



1. DMSO
2. Eribulin 10nM
3. Eribulin 50nM
4. BKM 0.5uM
5. RAD 0. 1uM
6. Eribulin 10Nm+ BKM 0.5uM
7. Eribulin 10nM+ RAD 0. 1uM
8. Eribulin 50nM+ BKM 0.5uM
9. Eribulin 50nM+ RAD 0. 1uM
10. BKM 0.5uM + RAD 0. 1uM
11. Eribulin 10nM + BKM 0.5uM + RAD 0. 1uM
12. Eribulin 50nM + BKM 0.5uM + RAD 0. 1uM
13. MDA468

Figure 4: MDA468 cells were treated with BKM

1.4 Rationale for Current Study

Based on our preclinical findings, we hypothesize that the combination of everolimus and eribulin will have a synergistic effect with a dual inhibition of both AKT and mTOR and will increase clinical activity in patients with metastatic TNBC. Previous studies of each of these drugs in MBC show acceptable toxicity, and we hypothesize that this drug combination could

offer a novel approach to treat patients with metastatic TNBC who are resistant to anthracyclines and taxanes or platinums.

2.0 Specific Objectives

The overall objective of this study is to conduct a phase I/Ib trial of everolimus plus eribulin in metastatic TNBC patients who are resistant to anthracyclines and/or taxanes or platinums. The trial will consist of a Phase I portion and a Phase Ib portion.

2.1 Primary Objective for Phase I Study

The primary objective of the Phase I portion of the study is to determine the safety and tolerability of everolimus and eribulin, and determine the recommended Phase Ib dose (RP2D) of the drug combination in patients with resistant metastatic TNBC.

2.2 Primary Objective for Phase Ib Study

The primary objective of the Phase IB portion of the study is to evaluate the event-free survival (EFS) rate for patients with resistant metastatic TNBC at the RP2D of everolimus and eribulin to determine if the drug combination is worthy of further study.

2.3 Secondary Objectives for Phase Ib Study

The secondary objectives of the Phase Ib portion of the study are to determine response rate, OS, toxicity, and PK for everolimus and eribulin in patients with resistant metastatic TNBC.

A further secondary objective is to collect blood, skin punch biopsies, and tumor biopsies before and after treatment from all patients and perform proteomic analysis to determine the level of inhibition of the PI3K pathway in tumor cells versus non-therapeutic targets.

3.0 Study Endpoints

3.1 Primary Endpoint

The primary endpoints of the study are:

- Phase I: DLT (see **Section 11** for statistical considerations).
- Phase IB: EFS at 4 months (see **Section 11** for statistical considerations).

3.2 Secondary Endpoints

The secondary endpoints are:

- Phase Ib: Response rate, PFS, OS, toxicity profile and PK parameters.

4.0 Study Design

This is a Phase I/Ib trial of everolimus plus eribulin in metastatic TNBC patients who are resistant to anthracyclines and/or taxanes or platinum.

4.1 Phase I Study

The Phase I portion of the trial will use the toxicity equivalence range (TEQR) design. Based on this design, the target equivalence range of dose-limiting toxicities (DLTs) is 0.20-0.35. Toxicity levels of ≥0.51 will be considered too toxic, and doses which achieve this level will be stopped. Patients will enter the protocol in cohorts of three. This phase will be considered complete when 12 patients are studied at a single dose level with a toxicity level <0.51. The RP2D will be the dose closest to the target of 0.25 below 0.51 based on isotonic regression. Based on these requirements, the TEQR design will use a minimum of 6 patients and a maximum of 24 patients (median of 18) to determine the MTD. (see **Section 11**).

The PMT team for protocol 14036 reviewed the data: dose level 2 was closed after 2 of 3 evaluable participants experienced DLTs an observed rate of 67%; dose level 1, we saw 4 DLTs in 8 evaluable participants giving an observed DLT rate of 50% on this dose. Our equivalence range for DLT rate for this trial is 20-35%, our observed rate of 50% is above 35% so we plan to de-escalate. Two participants experienced grade 4 neutrophil count decrease and 2 experienced mucositis (1 grade 3 DLT, 1 participant received 71% of Everolimus due to grade 2 which is a DLT). Thus, the team decided to amend the protocol to allow for two new dose levels at a lower dose of Eribulin, and start the next cohort of patients at dose level B1.

Dosing Schedule: cycle length is 21 days

Dose level A	Everolimus (oral)	Eribulin (intravenous)
A-1	2.5mg daily (day 1 to 21)	1.4mg/m^2 on days 1, 8
A1	5mg daily (day 1 to 21)	1.4mg/m^2 on days 1,8
A2	7.5mg daily (day 1 to 21)	1.4mg/m^2 on days 1,8
A3	10mg daily (day 1 to 21)	1.4mg/m^2 on days 1, 8

Dose level B	Everolimus (oral)	Eribulin (intravenous)
B1	5mg daily (day 1 to 21)	1.1mg/m^2 on days 1,8
B-1	2.5mg daily (day 1 to 21)	1.1mg/m^2 on days 1,8

4.2 Phase Ib Study

In the Phase Ib portion of the trial, a Simon's Optimal two-stage design will be used to determine if the combination of everolimus and eribulin results in a greater rate of patients who are event-free at 4 months than with eribulin alone. Based on data from the EMBRACE trial, we set the 4-month EFS rate for eribulin at 45%. We want to determine if the drug combination has a 4-month EFS rate of ≥70% (25% improvement). Setting both the type I and type II errors to 0.1 after 12 patients (from the MTD at the end of the phase I trial), if ≤5 patients are event-free at 4 months, the study will stop for further accrual. If ≥6 patients are event-free at 4 months we will enroll 15 more patients for a total of 27 patients at the RP2D. If ≥16 patients out of the 27 are

event-free at 4 months we will consider the combination worthy of further study (see **Section 11**).

Patient will be asked to provide a fresh tumor tissue from a newly obtained core or excisional biopsy of a tumor lesion. Newly –obtained is defined as a specimen obtained up to 6 weeks (42 days) prior to initiation of treatment on day 1. Subjects for whom newly-obtained samples cannot be provided (e.g. inaccessible or subject safety concern) may submit an archived specimen only upon agreement from the study PI.

For patients who consented for optional tumor biopsy upon disease progression, a sample of tumor tissue that may be biopsied or resected will also be collected (see **Section 12.2**).

To reduce dose-limiting oral mucositis, a **mandatory dexamethasone mouth wash (**alcohol-free 0.5 mg/5 mL dexamethasone solution**) will be used for primary prophylaxis of oral mucositis (Section 8.1.2)**.

We will assess tumor response using response evaluation criteria in solid tumors (RECIST) Version 1.1 criteria based on radiologic imaging every 2 cycles of treatment (6 weeks).

Toxicity will be evaluated based on the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) v4.0.

Patients enrolled in the expanded cohort at the RP2D will also be asked to undergo serial blood sampling to evaluate the PK of everolimus and eribulin. Although it is unlikely that these two agents will interact negatively, both drugs are substrates for the membrane transporter P-glycoprotein. Therefore, PK studies will be performed in the expanded cohort treated at the RP2D to evaluate the PK of the drug combination (see **Section 12.1**).

For correlative studies, blood will be collected from patients prior to the first dose of everolimus and eribulin, at 4 and 8 hours after the first dose of everolimus and eribulin on day 1, and at two post-dose time points on days 4 and 8 of the first cycle for patients enrolled on the expanded cohort of RP2D and consented for additional research blood collection.

Two sets of optional skin punch biopsies (2 biopsies per set for a total of 4 biopsies) will be performed for patients who consented for skin biopsy for the expanded cohort level.

- First set
    - Biopsy 1 (3mm diameter) will be performed one week prior to any dosing.

- Biopsy 2 (3mm diameter) will be performed on the same site as Biopsy 1 on the day of the first dose of eribulin and everolimus; cycle 1 (prior to any dosing).

- Second set
  - Biopsy 3 (3mm diameter) will be performed on a new site on the day of the first dose of eribulin and everolimus; cycle 1 (prior to any dosing).
  - Biopsy 4 (3mm diameter) will be performed on the same site as Biopsy 3, one week later on the day that the second dose of eribulin is given (prior to dosing).

Patient will be asked to provide a fresh tumor tissue from a newly obtained core or excisional biopsy of a tumor lesion prior to study entry. Newly –obtained is defined as a specimen obtained up to 6 weeks (42 days) prior to initiation of treatment on day 1. Subjects for whom newly-obtained samples cannot be provided (e.g. inaccessible or subject safety concern) may submit an archived specimen only upon agreement from the study PI.

For patients who consented for optional tumor biopsy upon progression, a sample of tumor tissue that may be biopsied or resected and will also be collected (see **Section 12.2**).

Serum, peripheral blood mononuclear cells (PBMC), skin biopsy tissue and tumor tissue will be processed and stored according to established laboratory protocols and stored at -80°C until proteomic analysis. Analysis will be performed by Luminex and mass spectrometry, and western blotting for pAKT (Ser473), and AKT, p4E-BP1 (ser65/Thr70) and 4E-BP1, pS6K1 (Thr389) and S6K1, and pS6 (Ser235/236) and S6 (see **Section 12.2**).

5.0 Patient Eligibility

5.1 Inclusion Criteria

- Patients must have histologically confirmed stage IV TNBC (patients who had metastatic disease within 6 months of lumpectomy or mastectomy for treatment of TNBC may be excused from repeat biopsy).
- Be willing to provide tissue from a newly obtained core or excisional biopsy of a tumor lesion. Newly –obtained is defined as a specimen obtained up to 6 weeks (42 days) prior to initiation of treatment on day 1. Subjects for whom newly-obtained samples cannot be provided (e.g. inaccessible or subject safety concern) may submit an archived specimen only upon agreement from the study PI.
- Patients must have had prior treatment with antracyclines and/or taxanes (resistant) or platinum including adjuvant or neoadjuvant therapy.
- Both measurable as well as non-measurable disease by RECIST 1.1 will be allowed.
- Patients with chemotherapy for metastatic disease (patients with 0-3 prior lines of chemotherapy for MBC).
- Age ≥18 years.
- Life expectancy of ≥ 3 months.

- Performance status: ECOG PS 0-2.
- Adequate bone marrow, liver and renal function as assessed by the following: Hemoglobin ≥9.0g/dl, absolute neutrophil count (ANC) ≥1,500/mm^3, Platelet count ≥100,000/mm^3, Creatinine ≤1.5 times the ULN Liver Function Tests: Total bilirubin less ≤ to 1 times ULN, ALT and AST ≤ to 2.5 times the ULN if no liver metastases. For patients with known liver metastases, AST and ALT must be ≤ to 5 times the ULN.
- Women of child-bearing potential must agree to use adequate contraception (hormonal or barrier method of birth control or abstinence) prior to study entry and for up to 8 weeks after ending treatment. Should a woman become pregnant or suspect that she is pregnant while participating on the trial, she should inform her treating physician immediately (see **Section 5.2**).
- Ability to understand and the willingness to sign a written informed consent document.
- Be willing to use dexamethasone mouth wash as directed.

5.2 Exclusion Criteria

- Patients who have had chemotherapy or radiotherapy within 2-weeks prior to entering the study or those who have not recovered from AEs due to agents administered >3 weeks prior to entering the study.
- Patients may not be receiving any other investigational agents.
- Patients with symptomatic brain metastases are excluded from this clinical trial.
- Uncontrolled current illness including, but not limited to, ongoing or active infection (>Grade 2 based on the NCI CTCAE v4.0, symptomatic congestive heart failure, unstable angina pectoris, myocardial infarction within the past 6 months, cardiac ventricular arrhythmias requiring anti-arrhythmic therapy, or psychiatric illness/social situations that would limit compliance with study requirements.
- Pregnant women.

- Prior eribulin use.
- Patients with HIV, chronic hepatitis B, or chronic hepatitis C (known from the existing medical record).
- Concomitant use with strong or moderate CPY3A4/PgP inhibitors and CPY3A4/PgP inducers.
- Women of child-bearing potential (WOCBP), defined as all women physiologically capable of becoming pregnant, must use highly effective methods of contraception during the study and 8 weeks after ending treatment. Highly effective contraception methods include combination of any two of the following:
    - Use of oral, injected or implanted hormonal methods of contraception or;
    - Placement of an intrauterine device (IUD) or intrauterine system (IUS);
    - Barrier methods of contraception: condom or occlusive cap (diaphragm or cervical/vault caps) with spermicidal foam/gel/film/cream/ vaginal suppository;
    - Total abstinence;
    - Male/female sterilization.

Women are considered post-menopausal and not of child-bearing potential if they have had 12 months of natural (spontaneous) amenorrhea with an appropriate clinical profile (e.g. age appropriate, history of vasomotor symptoms) or have had surgical bilateral oophorectomy (with or without hysterectomy) or tubal ligation at least six weeks prior to randomization. In the case of oophorectomy alone, only when the reproductive status of the woman has been confirmed by follow up hormone level assessment is she considered not of child-bearing potential.

- Male patients whose sexual partner(s) are WOCBP who are not willing to use adequate contraception, during the study and for 8 weeks after the end of treatment.
- Noncomplaint with oral medication and/or dexamethasone mouth wash

5.3 Informed Consent

All subjects must have the ability to sign a written informed consent (see **Section 6.2** for informed consent process).

5.4 Inclusion of Women and Minorities

The study is open to patients of all races and ethnicities. However, given the cancer under study, only women will be recruited to the study.

6.0 Screening, Registration, and Follow-up Studies

6.1 Subject Identification and Recruitment

Patients with histologically-confirmed stage IV resistant metastatic TNBC will be identified and asked to participate in the study by the clinician. All patients will be recruited from patients undergoing treatment at City of Hope (COH).

Diagnostic or laboratory studies performed exclusively to determine eligibility for this trial will be performed only after obtaining written informed consent (see **Section 6.2**). Studies or procedures performed for clinical indications (not exclusively to determine study eligibility) may be used for baseline values, even if the studies were done before informed consent was obtained (see **Study Calendar** for further details of study-related tests; **Section 6.4**).

6.2 Informed Consent Process

Patients will be given copies of the consent form and the patient bill of rights to review and sign if they consent to participate in the study. The Principal Investigator or Co-Investigators will explain the nature, duration, purpose of the study, potential risks, alternatives, and potential benefits, and all other information contained in the informed consent document. In addition, they will review the experimental subject's bill of rights and the Health Insurance Portability and Accountability Act (HIPAA) research authorization form. Research subjects will be informed that they may withdraw from the study at any time and for any reason without prejudice, including as applicable, their current or future care or employment at COH or any relationship they have with COH. General questions will be answered by the Principal Investigator and Co-Investigators, and more complex questions requiring a medical opinion will be answered by the Principal Investigator and Co-Investigator physicians.

Should sufficient doubt be raised regarding the adequacy of comprehension, further clarifications will be made and the questionnaire repeated until a satisfactory result is obtained. Prospective research subjects who cannot adequately comprehend the fundamental aspects of the research study with a reasonable amount of discussion, education, and proctoring will be ineligible for enrollment.

6.3 Dose Level Assignment

Dose level will be assigned as described in **Section 11**.

6.4 Study Calendar

ERIBULIN/EVEROLIMUS SCHEDULE Q21 DAYS																										
		Cycle 1												Cycle 2 +												
	Pre-Study[i]	Day1+/-3days	Day 2	Day 3	Day 4	Day 6	Day 7	Day 8+/-3days	Day 9	Day 12	Day 15[i]	Day 16	Day 21	Day 1+/-3days	Day 2[i]	Day 3	Day 4	Day 6	Day 7	Day 8+/-3days	Day 11	Day 12	Day 15	Day 16	Day 21[j]	Off Study[i]
Everolimus		←……. Continuous Day 1 through 21…….→												← …….Continuous Day 1 through 21…….→												
Eribulin[a]		X						X						X						X						
Informed consent	X																									
Demographics	X																									
Medical history	X																									
Height	X																									
EKG [b]	X																									
B-HCG [c]	X																									
Con-Meds	X	X---------- ----------X																								X
Everolimus dose diary		X----------X																								X
Physical exam	X	X												X												X
Vital signs	X	X												X												X
Weight	X	X												X												X
Performance status	X	X												X												X
CBC w/diff, plts	X	X						X						X						X						X
Serum chemistry [d]	X	X												X												X
Tumor Biopsy [i]	X																									X
Skin Punch Biopsy [e]	X	X						X																		
Blood/Correlative [h]	X	X			X			X																		
Pharmacokinetics[f]		X	X											X	X	X	X			X						
Adverse events		X---------- ----------X																								X
Tumor measurements	X	**Tumor measurements are repeated every 2 cycles (~6 weeks). Documentation (radiologic) must be provided for patients removed from study for progressive disease.**																								X
Radiologic	X	**Radiologic measurements are repeated every 2 cycles (~6 weeks).**																								X

evaluation[g]			

a: Actual body weight will be used for BSA and Eribulin dose calculation. b: Pre-study and every two cycles. **c:** Serum pregnancy test (women of childbearing potential). **d:** Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus, potassium, magnesium, total protein, SGOT [AST], SGPT [ALT], sodium. **e:** Two sets of punch biopsies (two biopsies per set for a total of four biopsies – Optional for patients who consented at RP2D level) will be performed as follows: First set - Biopsy 1 will be performed one week prior to any dosing (pre-study). Biopsy 2 will be performed on the same site as Biopsy 1 on the day of the first dose of eribulin and everolimus; cycle 1 (prior to any dosing). Second set: Biopsy 3 will be performed on a new site on the day of the first dose of eribulin and everolimus; cycle 1 (prior to any dosing). Biopsy 4 will be performed on the same site as Biopsy 3, one week later on the day that the second dose of eribulin is given (prior to dosing). **f:** To be performed in a subset of patients enrolled on the Phase Ib portion of the study (see section 12.0). **g:** Staging images (CT scans, bone scan) must be performed within 28 days prior to study entry. **h:** Blood correlative studies will be collected pre-study, before the first dose of everolimus and eribulin, at 4 and 8 hours after the first dose of everolimus and eribulin on day 1, and at two post-dose time points on days 4 and 8 of the first cycle for patients on the expanded cohort of RP2D. i. Mandatory tumor biopsy specimen (fresh frozen and/or paraffin-embeded) will be obtained prior to study entry. An optional tumor tissue biopsy will be obtained upon disease progression. j: Pre-study assessments should be performed within 28 days prior to initiating therapy, unless otherwise noted. For the on-study treatment, there is a +/- 3 days of window allowed.

Items e, f, h: ONLY for patients enrolled to the expanded cohort, i.e. RP2D.

6.5 Registration Guidelines

General Guidelines

Eligible patients will be registered on the study centrally at the Data Coordinating Center (DCC) at the City of Hope. Staff (including physicians, protocol nurses and/or CRAs) should call the DCC at (626) 256-4673, ext. 64267 to verify dose level and slot availability, and to reserve a slot for a specific prospective subject. Slots can only be held for a limited time as specified in Appendix C.

Eligible patients must be registered within 2 weeks prior to start of protocol therapy.

Issues that would cause treatment delays should be discussed with the Principal Investigator. If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled. The Data Coordinating Center should be notified of cancellations as soon as possible.

Registration Process

Once a slot at a dose level has been reserved, the signed informed consent has been obtained, all pretreatment evaluations have been performed, and patient's eligibility has been confirmed by the Data Coordinating Center, a patient will be registered on study.

To register a patient, the treating physician should contact the protocol nurse or the responsible Clinical Research Associate (CRA) in Clinical Trial Office (CTO) to complete the eligibility/registration form. The protocol nurse or CRA will contact the Data Coordinating Center at the City of Hope (626-256-4673, ext. 64267 or e-mail dcc@coh.org), EMAIL a copy of the completed eligibility checklist, required pre-study tests (per protocol – and may include laboratory, CT and pathology reports), signed Informed Consent, signed Patients' Bill of Rights and HIPAA authorization form to dcc@coh.org. See Appendix C ("Registration Procedures").

The protocol nurse or CRA will then call the Data Coordinating Center (626-

256-4673 ext. 64267) to confirm receipt of all registration documents. To complete the registration process, the Data Coordinating Center will:

- Verify and confirm the patient's eligibility.
- Assign a patient accession number (for example, COH-001, COH-002, etc.).
- Register the patient on study centrally (the City of Hope CRA assigned to the trial will still be responsible for accessioning via MIDAS). If multi-site study, COH DCC staff will be responsible for registering the participating site patients at City of Hope.
- Assign the patient to a dose level (as applicable).
- Complete and email a Confirmation of Registration form within 24 hours to include the COH patient study number and dose level (as applicable) to the study team, which will include the Principal Investigator, treating physician, protocol nurse, CRA and COH IDS Pharmacy.
- Call the protocol nurse and/or CRA to verbally confirm registration.
- A patient failing to meet all protocol requirements will not be registered.

7.0 Study Treatment Protocol

7.1 Eribulin mesylate (E7389)

7.1.1 Nomenclature

Chemical Name (USAN/INN):
(2R,3R,3aS,7R,8aS,9S,10aR,11S,12R,13aR,13bS,15S,18S,21S,24S,26R,28R,29aS)-2-[(2S)-3-Amino-2-hydroxypropyl]-3-methoxy-26-methyl-20,27-dimethylidenehexacosahydro-11,15:18,21:24,28-triepoxy-7,9-ethano-12,15-methano-9H,15H-furo[3,2-i]furo[2',3':5,6]pyrano[4,3-b][1,4]dioxacyclopentacosin-5(4H)-one methanesulfonate (salt).

7.1.2 Chemical and structural formula

Molecular formula: C41H63NO14S (C40H59NO11・CH4O3S).

Molecular weight: 826.00.

7.1.3 Physical and chemical characteristics

Appearance: White powder.

Hygroscopicity: Eribulin is hygroscopic.

7.1.4 Description

Eribulin currently used in clinical studies contains 1.0mg drug substance in 2.0mL of solution. Eribulin rug product is a clear, colorless, and sterile solution packaged in a glass vial.

7.1.5 Storage

The long-term storage condition for commercial Eribulin is 25°C (do not freeze). Investigational labeled Eribulin is stored in the refrigerator at 2-8 °C. .

7.1.6 Dosage

The drug product will provided by Eisai for this trial. It will be administered per Eisai's directions without dilution or diluted in up to 100mL 0.9% sodium chloride (for injection). Do not dilute in or administer through an intravenous line containing solutions with dextrose.

7.2 Everolimus

7.2.1 Nomenclature

Chemical name: (1R,9S,12S,15R,16E,18R,19R,21R,23S,24E,26E,28E,30S,32S,35R) -1,18-dihydroxy-12-[1]-19,30-dimethoxy15,17,21,23,29,35-hexamethyl-11,36-dioxa-4-azatricyclo[30.3.1.04,9]hexatriaconta-16,24, 26,28-tetraene-2,3,10,14,20pentaone

7.2.2 Chemical and structural formula

Molecular formula: C53H83NO14.

Molecular weight: 958.2.

7.2.3 Physical and chemical characteristics

Appearance: White to faintly yellow powder.

7.2.4 Description

Everolimus stabilized with butylated hydroxytoluene (BHT) is amorphous, and contains 0.2% BHT as an antioxidant. The drug substance, everolimus, contains 15 asymmetric carbon atoms and 4 substituted double bonds. The configuration of the asymmetric carbon atoms and the double bonds are guaranteed by the microbial origin of rapamycin, the starting material of the synthesis, and by the X-ray analysis performed on crystalline everolimus. The configuration at carbon 40 is not changed by the chemical derivatization that converts rapamycin into everolimus.

7.2.5 Storage

Stability: Shelf-life of 36 months when stored at <30°C. Protect from light and moisture.

7.2.6 Dosage

2.5mg and 5mg tablets will be provided by Novartis for this trial. Everolimus should be administered orally, once daily at the same time every day either consistently with food or consistently without food. Tablets should be swallowed whole with a glass of water. The tablets must not be chewed or crushed. For patients unable to swallow tablets, everolimus tablet(s) should be dispersed completely in a glass of water (containing approximately 30mL) by gently stirring, immediately prior to drinking. The glass should be rinsed with the same volume of water and the rinse should be completely swallowed to ensure that the entire dose is administered.

7.3 Treatment Overview

For a tabular view of the treatment, monitoring, and follow-up schedule, see **study calendar** in **Section 6.4**. For dose levels see the study design (**Section 4.0**).

7.4 Planned Duration of Therapy

Treatment cycle length is 21 days with response evaluation every 2 cycles.

7.5 Subject Follow-Up

Patients will be followed after removal from study until death. Patients removed from study for AEs will be followed until resolution or stabilization of the AE (See **Section 10** for further AE information).

7.6 Supportive Care, Other Concomitant Therapy, Prohibited Medications

Ondansetron will be used as pre-medication for prophylaxis of eribulin induced nausea. If needed, prior to each treatment with eribulin, patients may be pre-medicated with dexamethasone 10mg to prevent a hypersensitivity reaction. Ondansetron 8mg oral tablet will be used as needed at home for treatment related nausea and/or vomiting.

7.6.1 Dexamethasone Mouth Wash

To reduce dose-limiting oral mucositis, a **mandatory dexamethasone mouth wash (**alcohol-free 0.5 mg/5 mL dexamethasone solution**) will be used for primary prophylaxis of oral mucositis**. Patients will start the prophylactic oral care regimen on the same day they start treatment with everolimus + eribuline. The mouthwash should be taken as directed in section **8.1.2.** Treating physician or other health provider of the treatment team will write the prescription of the steroid-based mouthwash. Usage of the mouth wash should be documented by patient and followed up during clinic visit.After first two cycles of treatment, patient can use the steroid mouth wash 4 times a day as needed .

7.6.2 Concomitant medications

Patients must be instructed not to take any medications (over-the-counter or other products) during the protocol treatment period without prior consultation with the investigator. The investigator should instruct the patient to notify the study site about any new medications he/she takes after the start of study drug. All medications (other than study drug) and significant non-drug therapies (including physical therapy and blood transfusions) taken within 28 days of starting study treatment through the 30-day safety follow up visit should be reported on the CRF.

7.6.2.1 Angioedema with concomitant use of angiotensin-converting enzyme (ACE) inhibitors

Patients taking concomitant ACE inhibitor therapy may be at increased risk for angioedema (e.g. swelling of the airways or tongue, with or without respiratory impairment).

7.6.2.2 Cytochrome P450 and P-glycoprotein inhibitors/inducers/substrates

Everolimus is metabolized by CYP3A4 in the liver and to some extent in the intestinal wall. Inhibitors of CYP3A4 or PgP may cause increased everolimus concentrations. Inducers of CYP3A4 or PgP may cause decreased everolimus concentrations. Everolimus is a substrate of CYP3A4, and a substrate and moderate inhibitor of the multidrug efflux pump, PgP (PgP, MDR1, and ABCB1). The extent of absorption and subsequent elimination of systemically absorbed everolimus may be influenced by products that are substrates, inhibitors, or inducers of CYP3A4 and/or PgP. In vitro studies showed that everolimus is a competitive inhibitor of CYP3A4 and of CYP2D6, potentially increasing the concentrations of products eliminated by these enzymes.

Therefore,

- Co-administration with strong inhibitors of CYP3A4 or with a P-glycoprotein (PgP) inhibitor is not permitted.
- Coadministration with PgP substrates should be with caution as everolimus can affect their drug concentrations.
- Co-administration with moderate CYP3A4 inhibitors is not permitted. If a patient requires co-administration of moderate CYP3A4 inhibitors, everolimus therapy must be interrupted. Once the inhibitor is discontinued, everolimus can be resumed only after discussion with the PI and a after a minimum washout period of 2 days (or longer depending on agent's half life).
- Grapefruit, Seville oranges, and starfruit affect P450 and PgP activity. Concomitant use is not permitted.

- Coadministration of a CYP3A4 inducer is not permitted except topical steroid. Oral dexamethasone mouth wash is allowed.
- Coadministration with CYP3A4 and CYP2D6 substrates should be done with caution.

For a current table of Substrates, Inhibitors and Inducers please access the following website: http://www.fda.gov/Drugs/DevelopmentApprovalProcess/DevelopmentResources/DrugInteractionsLabeling/ucm093664.htm.

Please refer to the tables below listing relevant inducers and inhibitors of CYP3A and for a list of relevant substrates, inducers, and inhibitors of PgP.

Clinically relevant drug interactions: inducers, and inhibitors of isoenzyme CYP3A

Inducers
mitotane, genistein, thioridazine, bexarotene, clobazam, danshen, Echinacea, garlic (allium sativum), ginkgo (ginkgo biloba), glycyrrhizin, methylprednisolone, primidone, raltegravir, sorafenib, telaprevir, terbinafine, carbamazepine, glucocorticoids, modafinil, oxcarbazepine, phenobarbital, phenytoin, pioglitazone, rifabutin, rifampin, St. John's wort, troglitazone, efavirenz, nevirapine, topiramate, avasimibe, bosentan, etravirine, nafcillin, ritonavir, talviraline (not available in US market), tipranavir, amprenavir, aprepitant, armodafinil (R-modafinil), dexamethasone*, nevirapine, prednisone, pleconaril (not available in US market), rufinamide
Inhibitors
Strong inhibitors: Boceprevir, cobicistat, telaprevir,clarithromycin, conivaptan, indinavir, itraconazole, ketoconazole, lopinavir, mibefradil, nefazodone, nelfinavir, ritonavir, saquinavir, telithromycin, troleandamycin, voriconazole, tipranavir, elvitegravir, posaconazole. Moderate inhibitors: Aprenavir, cyclosporine, schisandra sphenanthera, aprepitant, atazanavir, casopitant, cimetidine, ciprofloxacin, darunavir, diltiazem, erythromycin, fluconazole, grapefruit juice (citrus parasidi fruit juice), imatinib, tofisopam, verapamil, amprenavir, fosamprenavir, dronedarone.

***Topical steroid such as oral dexamethasone mouth wash is allowed.**

Clinically relevant drug interactions: substrates, inducers, inhibitors of PgP and PgP/CYP3A dual inhibitors

Substrates
Talinolol, everolimus, digoxin, fexofenadine, indinavir, vincristine, colchicine, topotecan, paclitaxel
Inducers
rifampin, St John's wort

PgP Inhibitors and PgP/CYP3A Dual Inhibitors
Azithromycin, fluvoxamine, nelfinavir, paroxetine, amiodarone, captopril, carvedilol, clarithromycin, conivaptan, diltiazem, dronedarone, elacridar, erythromycin, felodipine, fexofenadine, ginkgo (ginkgo biloba), indinavir, itraconazole, , lopinavir, mibefradil, milk thistle (silybum marianum), nifedipine, nitrendipine, quercetin, quinidine, ranolazine, ritonavir, saquinavir, schisandra chinensis, St John's wort (hypericum perforatum), talinolol, telmisartan, tipranavir, valspodar, verapamil.
Reference: Internal Clinical Pharmacology Drug-drug interaction (DDI) memo, updated Oct. 2, 2011, which summarizes DDI data from three sources including the FDA's "Guidance for Industry, Drug Interaction Studies", the University of Washington's Drug Interaction Database, and Indiana University School of Medicine's Drug Interaction Table.

7.6.3 Vaccinations

Immunosuppressants may affect the response to vaccination and vaccination during treatment with everolimus may therefore be less effective. The use of live vaccines should be avoided during treatment with everolimus. Examples of live vaccines are: intranasal influenza, measles, mumps, rubella, oral polio, BCG, yellow fever, varicella, and TY21a typhoid vaccines.

8.0 Dose Delays/Modifications

8.1 Everolimus

The most common AEs reported for everolimus that may require dose modifications are stomatitis, rash, and non-infectious pneumonitis.[30]

8.1.1 Dose Limiting Toxicities

Definition of dose limiting toxicities (DLTs) based on CTCAE 4.0. All toxicities will be attributed to study drug (everolimus) except hematological toxicities. Hematological DLTs will be defined for the combination of eribulin and everolimus. If a hematological DLT occurs, dose reduction or delay will be conducted according to rules defined in 8.1.6 and 8.2.2.

DLT is defined as an AE or abnormal laboratory value as at least possibly related to the study medication and meets any of the criteria per CTCAE 4.0 . The following table is recommended for the most anticipated toxicities.

Decisions to escalate, de-escalate, or expand a dose level will be based on the first course of treatment. To be evaluable for toxicity, a patient must receive at least 1 complete course of treatment (at least 75% of planned dose of both drugs without toxicity) and be followed for at least 21 days (more if there is a dose delay) or have experienced a DLT as defined above. All patients enrolled are to be fully followed for toxicity; any patients who

are not evaluable for toxicity will be replaced. Intra-patient dose escalation is not allowed.

Table 8.1.1a Criteria for defining dose-limiting toxicities.

Toxicity	DLT criteria
Hematology	CTCAE grade ≥3 neuropenia lasting more than 7 consecutive days
	CTCAE grade 4 thrombocytopenia
	CTCAE grade 4 neutropenia
	CTCAE Grade 3 neutropenia with fever (temperature ≥38.5C)
Non-hematology	Any CTCAE grade ≥3 AEs with the following exceptions
Exceptions to DLT criteria	Grade 3 alopecia
	Controllable grade 3 nausea and vomiting
	<5 days of CTCAE grade 3 fatigue
	Triglycerides < 1,500mg/dl, which recover within 1 week
	Grade 3 laboratory abnormalities that are correctable to grade 2 or less within 24 hours
	Grade 3 hyperglycemia that is controlled to Grade 2 and under should not be considered DLT. For management of hyperglycemia, please refer to section 8.1.9.
Treatment delays	>2 weeks as a result of unresolved toxicity during the first cycle of therapy (failure of ANC to recover to ≥1,000/μL or platelets to recover to ≥50,000/μL within 14 days of the last treatment)
Others	Failure to complete at least 75% of planned dose of either one of the drugs during cycle 1, due to toxicity, will also be considered a DLT.

Follow-up for toxicities.
Patients whose treatment is interrupted or permanently discontinued due to an AE or clinically significant laboratory value, must be followed up weekly (or more frequently if clinically indicated) for 4 weeks, and subsequently at approximately 4 week intervals, until resolution or stabilization of the event, whichever comes first. Appropriate clinical experts such as cardiologist, pulmonologists etc should be consulted as deemed necessary. All patients must be followed up for 21 days when taken off study .

8.1.2 Stomatitis

Inflammation of the mucous membranes in the mouth may occur. Ulcerated areas in the oral cavity, inner surface of the lips, or tongue, closely resembling aphthous stomatitis may occur. Onset typically occurs early (≤1 month after treatment initiation) and is

transient. 44%-70% of everolimus-treated patients develop mouth ulcers, stomatitis, or oral mucositis, which are mostly Grade 1 or 2.

To reduce dose-limiting oral mucositis, a **mandatory dexamethasone mouth wash (**alcohol-free 0.5 mg/5 mL dexamethasone solution**) will be used for primary prophylaxis of oral mucositis**. Steroid based mouth wash was studied in a randomized phase II study evaluating miracle mouth wash plus hydrocortisone versus prednisolone mouth rinses as prophylaxis for everolimus- associated stomatitis[37]. This study provided evidence of a reduced incidence of mTOR-associated stomatitis with prophylactic use of a steroid mouth rinse. The 25% incidence of all-grade and 8%/0% incidence of G2/3 stomatitis compare favorably with the 67% and 24%/8% incidence of all-grade and G2/3 stomatitis, respectively, in BOLERO-2. These preliminary data also demonstrated the safety and tolerability of these 2 steroid mouth rinses.

Currently, there is an ongoing phase II trial examining the effect of dexamethasone based mouth wash[38]. We would like to utilize this dexamethasone based mouth wash for the current study. The mouth care regimen consists of a steroid-based mouthwash (alcohol-free 0.5 mg/5 mL dexamethasone solution) . Patients will start the prophylactic oral care regimen on the same day they start treatment with everolimus + eribuline. The mouthwash should be taken as follow:

- Swish and spit 10ml of mouthwash 4 times each day
- Hold mouthwash in mouth for a minimum of 2 minutes
- Swish it around in the mouth, so it comes in contact with every surface of the mouth
- Spit it out (do not swallow)
- Abstain from eating or drinking for a leat 1 hour after performing mouthwash regimen
- Continue with assigned oral care regimen for the first 2 months (56 days) of everolimus + eribuline therapy, after with mouthwash will be stopped.

Treating physician or other health provider of the treatment team will write the prescription of the steroid-based mouthwash. Usage of the mouth wash should be documented by patient and followed up during clinic visit.**After first two cycles of treatment, patient can use the steroid mouth wash 4 times a day as needed .**

Upon diagnosis of ≥ grade 1 stomatitis, a normal saline (0.9% sodium chloride) mouth rinse will be added while patient is continuing the dexamethasone mouthwash. The normal saline is administered as a 10mL swish and spit (QID) (must be done prior to the steroid mouthwash QID). Patients administered normal saline (0.9% sodium chloride) mouth rinse (swish and spit), then waited for 10-15 mins, and then administered dexamethasone mouth wash. No dose adjustment is required for grade I stomatitis.

Dose adjustments for stomatitis

Grade	Symptom	Management	Everolimus Dose Adjustment
2	Symptomatic but can eat and swallow a modified diet.	• Manage with topical analgesic mouth treatment (e.g. benzocaine, butyl aminobenzoate, tetracaine hydrochloride, menthol or phenol) with or without topical corticosteroids (i.e. triamcinolone oral paste). • Avoid agents containing alcohol, hydrogen peroxide, iodine and thyme derivatives.	• Interrupt everolimus administration until resolution to ≤grade 1 or baseline grade / value. • If resolution occurs within ≤7 days, everolimus should be re-started at the dose level prior to interruption. • If resolution takes >7 days, or if event recurs within 28 days, hold everolimus until recovery to ≤ grade 1 or baseline grade / value and reintroduce everolimus at one dose level lower, if available. • Patients will be withdrawn from the study if they fail to recover to ≤ grade 1 or baseline grade / value within 28 days.
3	Symptomatic and unable to adequately eat or hydrate orally.	Same as above.	• Temporary dose interruptions until recovery to Grade ≤1. Re-initiate everolimus at a lower dose.
4	Severe (symptoms are life threatening).		• Discontinue everolimus and treat with appropriate medical therapy.

8.13 Rash

Rash is reported in 29%-59% of patients treated with everolimus. It may be erythematous maculopapular, acneiform, and eczematoid. Research participants should receive the following education about rashes:

- Inform research participant of the possibility of developing rash.
- Inform research participant to promptly report any signs or symptoms of rash to initiate treatment early.
- Educate research participants to employ good skin care including:
  - Moisturize frequently. Use a thick, alcohol-free emollient cream.
  - Take short lukewarm showers, using mild, moisturizing (fragrance-free) soap.

- Bathe in lukewarm water plus 1-2 cups of baking soda.
- Use a sunscreen of SPF 15 or higher, preferably containing zinc oxide or titanium dioxide (minimize sun exposure).

Dose adjustments for rash

Grade	Symptoms	Management	Everolimus Dose Modification
2	Moderate	• Consider treatment with topical steroids, topical antibiotics and/or oral antihistamines	• Temporary dose interruptions until recovery to Grade ≤1. • Re-initiate everolimus at the same dose. • If rash recurs at Grade 2, interrupt dose until recover to Grade ≤1. Re-initiate everolimus at a lower dose.
3 (or intolerable grade 2 papulopustular)	Severe	• Consider oral antibiotics	• Temporary dose interruptions until recovery to Grade ≤1. Re-initiate everolimus at a lower dose.
4	Life-Threatening		• Discontinue everolimus and treat with appropriate medical therapy.

8.14 Non-infectious pneumonitis

Non-infectious pneumonitis is reported in 14%-19% of patients treated with everolimus. Incidence of Grade 3 and 4 non-infectious pneumonitis has been reported up to 4% and up to 0.2%, of patients respectively. Fatal outcomes have been observed.

Dose adjustments for non-infectious pneumonitis

Grade	Symptom	Management	Everolimus Dose Modification
2	Symptomatic, not interfering with ADL.	• Rule out infection. • Consider treatment with corticosteroids.	• Consider interruption of therapy until symptoms improve to Grade ≤1. • Re-initiate everolimus at a lower dose. • Discontinue treatment if failure to recover within 4 weeks.

3	Symptomatic, interfering with ADL, 0_2 required.	• Rule out infection. • Consider treatment with corticosteroids.	• Consider interruption of therapy until symptoms improve to Grade ≤1. • Re-initiate everolimus at a lower dose. If toxicity recurs a Grade 3, consider discontinuation.
4	Life threatening, ventilator support indicated.	• Rule out infection. • Consider treatment with corticosteroids.	• Discontinue everolimus.

8.15 Dosing guidelines for everolimus-related non-hematologic toxicities

Toxicity	**Action**
Non-Infectious Pneumonitis	Please refer to table above.
AST or ALT elevation Grade 1 (> ULN - 3.0 x ULN) Grade 2 (> 3.0 - 5.0 x ULN)	Maintain current dose level
AST or ALT elevation Grade 3 (>5.0 - 20.0 ULN)*	Interrupt everolimus administration until resolution to ≤grade 1 (or ≤grade 2 if baseline values were within the range of grade 2). If resolution occurs ≤7 days, everolimus should be re-started at the dose level prior to interruption. If resolution takes >7 days, or if event recurs within 28 days, hold everolimus until recovery to ≤grade 1 or baseline grade / value and reintroduce Everolimus at one dose level lower, if available.
AST or ALT elevation Grade 4 (>20 x ULN) Recurrence of grade 4 AST or ALT elevation after dose reduction or toxicity requiring Everolimus interruption for > 28 days	Interrupt everolimus administration until resolution to ≤grade 1 (or ≤ grade 2 if baseline values were within the range of grade 2). If resolution occurs ≤7 days, everolimus should be re-started at one dose level lower. If resolution takes >7 days, discontinue everolimus. Discontinue everolimus.
Intolerable grade 2 mucositis, or grade 3 AE, except hyperglycemia or hypertriglyceridemia or hypercholesterolemia	Interrupt everolimus administration until resolution to ≤grade 1 or baseline grade / value. If resolution occurs within ≤7 days, everolimus should be re-started at the dose level prior to interruption. If resolution takes >7 days, or if event recurs within 28 days, hold everolimus until recovery to ≤ grade 1 or baseline grade / value and reintroduce everolimus at one dose level lower, if available. Patients will be withdrawn from the study if they fail to recover to ≤ grade 1 or baseline grade / value within 28 days.

Toxicity	Action
Any other grade 4	Hold everolimus until recovery to grade ≤1 or baseline value. Reintroduce Everolimus at one dose level lower, if available.
Grade 3 or 4 clinical liver failure (asterixis or encephalopathy/coma)	Discontinue everolimus.
Recurrence of intolerable grade 2 mucositis or grade 3 event after dose reduction	Reduce dose to the next lower dose level, if available. The lowest possible dose level of everolimus is 2.5mg daily. Below this level, everolimus must be discontinued. If toxicity recurs at Grade 3, consider discontinuation.
Recurrence of grade 4 after dose reduction	Discontinue everolimus.
Any non-hematologic toxicity requiring Everolimus interruption for > 28 days	Discontinue everolimus.

8.1.6 Dosing guidelines for Everolimus related hematologic toxicities

Toxicity	Action
Grade 2 thrombocytopenia (platelets <75, ≥ 50x109/L)	No action
Grade 3 thrombocytopenia (platelets <50, ≥25 x 109/L)	Interrupt everolimus until resolution to grade ≤1 If resolution occurs ≤7 days, reintroduce everolimus at the dose level prior to interruption. If resolution occurs >7 days, or event occurs within 28 days, reintroduce everolimus at one dose level lower, if available.
Grade 4 thrombocytopenia (platelets <25 x 109/L)	Interrupt everolimus until recovery to grade ≤1. Then reintroduce everolimus at one dose level lower, if available.
Grade 3 neutropenia or anemia (neutrophil <1, ≥0.5 x 109/L)	Interrupt everolimus until resolution to grade ≤1 or baseline value If AE resolution occurs ≤7 days, reintroduce everolimus at the same dose level. If AE resolution occurs >7 days, or event occurs within 28 days, reintroduce everolimus at one dose level lower, if available.
Grade 4 neutropenia or anemia	Interrupt everolimus until recovery to grade ≤1 or baseline value. Reintroduce everolimus at one dose level lower, if available.*
Febrile neutropenia	Interrupt everolimus until resolution to grade ≤1 (or baseline value) and no fever. Reintroduce everolimus at one dose level lower, if available.*

Recurrence of grade 3 toxicity after dose reduction	Reduce dose to the next lower dose level, if available. The lowest possible dose level of everolimus is 5mg every other day (2.5 mg daily). Below this level, everolimus must be discontinued.
* Recurrence of grade 4 toxicity (including febrile neutropenia) after dose reduction	Discontinue everolimus
* Any hematologic toxicity requiring Everolimus interruption for > 28 days	Discontinue everolimus

8.1.7 Management of infections

Everolimus has immunosuppressive properties and may predispose patients to bacterial, fungal, viral or protozoal infections, including infections with opportunistic pathogens. Localized and systemic infections, including pneumonia, other bacterial infections, invasive fungal infections, such as aspergillosis or candidiasis and viral infections including reactivation of hepatitis B virus, have been described in patients taking everolimus Some of these infections have been severe (e.g. leading to sepsis, respiratory or hepatic failure) and occasionally have had a fatal outcome.

Physicians and patients should be aware of the increased risk of infection with everolimus. Treat pre-existing infections prior to starting treatment with everolimus. While taking everolimus, be vigilant for symptoms and signs of infection; if a diagnosis of infection is made, institute appropriate treatment promptly and consider interruption or discontinuation of everolimus.

If a diagnosis of invasive systemic fungal infection is made, discontinue everolimus and treat with appropriate antifungal therapy.

Cases of pneumocystis jirovecii pneumonia (PJP), some with a fatal outcome, have been reported in patients who received everolimus. PJP may be associated with concomitant use of corticosteroids or other immunosuppressive agents. Prophylaxis for PJP should be considered when concomitant use of corticosteroids or other immunosuppressive agents are required.

8.1.8 Management of diarrhea

Appearance of grade 1-2 diarrhea attributed to study drug toxicity may be treated with supportive care such as loperamide, initiated at the earliest onset (for example 4 mg orally followed by 2mg orally every 2 hours until resolution of diarrhea).

8.1.9 Management of hyperglycemia

Grade 3 hyperglycemia has been observed in patients receiving everolimus therapy. The fasting state of patients should be verified when interpreting results. It is suggested that optimal glucose control should be achieved before starting a patient on everolimus and

should be monitored during everolimus therapy. Should hyperglycemia develop during protocol therapy, standard glucose control interventions should be implemented. If hyperglycemia was well-controlled and improved to less or equal then grade II, this will not count toward a DLT.

8.2 Eribulin

8.2.1 Dose delays during therapy

Do not administer eribulin mesylate on Day 1 or Day 8 for any of the following:

- ANC $<1\times10^9$/L
- PLT $<75\times10^9$/L
- Grade 3 or 4 non-hematological toxicities attributed by eribulin.

8.2.2 Dose adjustment during therapy

Subjects should be clinically evaluated during treatment by physical examination and laboratory testing including complete blood counts as indicated per protocol (see **Section 6.4**). If Grade 3 and 4 toxicities are present, treatment should be delayed to allow recovery. Subjects should only be retreated when ANC is $\geq1\times10^9$/L and PLT are $\geq75\times10^9$/L and all other toxicity from a previous cycle has recovered to ≤Grade 2.

Recommended dose reductions as indicated in the US Package Insert are as follows:

Permanently reduce the 1.4mg/m^2 eribulin mesylate dose to 1.1mg/m^2 for any of the following:

- ANC <500 mm^3 for >7 days.
- ANC <1,000 mm^3 with fever or infection.
- Platelets <25,000 mm^3.
- Platelets <50,000 mm^3 requiring transfusion.
- Non-hematologic Grade 3 or 4 toxicities graded according to NCI CTCAE v4.0.
- Omission or delay of Day 8 dose in previous cycle for toxicity.

Furthermore:

- If any event requires permanent dose reduction while receiving 1.1mg/m^2, reduce the dose to 0.7mg/m^2.
- If any event requires permanent dose reduction while receiving 0.7mg/m^2, discontinue eribulin.

There will be no dose reductions outside of the scheduled physical examination and laboratory testing including complete blood counts indicated per protocol (see Section 6.4).

9.0 Discontinuation of Study Treatment

9.1 Patient-initiated Discontinuation of Study Treatment

Even after a patient agrees to take part in the study, she may stop therapy or withdraw from the study at any time. If the study participant stops treatment but still allows the study physician to submit follow-up information, she will continue to be followed clinically according to the study schedule. Alternatively, she may choose to have no further interaction regarding the study in which case the Principle Investigator or Co-Investigators must obtain written documentation as to the patient's decision to withdraw fully from the study.

9.2 Investigator-initiated Discontinuation of Study Treatment

The investigator may require a study subject to discontinue study treatment in the event of one of the following:

- The patient develops a serious AE (SAE) that she cannot tolerate or that cannot be controlled with other medication (see **Section 10**).
- The patient has disease progression.
- The patient has concurrent illness that prevents further administration of treatment

If study therapy is stopped but the study subject allows the physician to submit follow-up information, she will continue to be followed clinically according to the study schedule. A Withdrawal/Discontinuation Form will be completed by the Principal Investigator, which will allow determination of which study subjects are receiving off-protocol therapy.

10.0 Data Safety Monitoring

10.1 Definition of Risk Level

This is a Risk Level 3 study, as defined in the "Guidance, Policy and Procedures for Data and Safety Monitoring for In-House Trials at City of Hope". http://www.infosci.coh.org/prot_office/forms/Guidance.doc. Risks are at least balanced by the potential benefits to subjects and the importance of the knowledge that may result. Adverse events are expected and a formal plan for monitoring and reporting these AEs is required (outlined below).

10.2 Monitoring and Personnel Responsible for Monitoring

The Protocol Management Team (PMT) consisting of the Principle Investigator, Collaborating Investigators, Co-Investigators, CRA, protocol nurse, and statistician is responsible for monitoring the data and safety of this study, including implementation of any stopping rules for safety and efficacy.

This study will utilize the Phase I tracking log to monitor data and safety for dose escalation, recording doses administered, and resultant AEs. The tracking log will contain dose levels administered, DLT-defining AEs, and documentation that the data from a dose level is complete before dose escalation. Those data and safety elements will be reported to the COH DSMC as applicable within the PMT report, which will be submitted quarterly or semi-annually from the anniversary date of activation, as noted in **Table 1**. Protocol-specific data collection will include the following items: patient demographics, toxicities summarized by dose, course, organ and severity and best overall response.

Table 1: City of Hope PMT Reporting Timelines for the DSMC

Risk Level	Phase	Standard Reporting Requirement
RL 1, RL2, and Compassionate Use Studies	No reports required	
3	I	Every 3 months from activation date, as indicated in MIDAS
3	Pilot, Feasibility, II-IV	Every 6 months from activation date, as indicated in MIDAS
4	Pilot, Feasibility, I-IV	Every 3 months from activation date, as indicated in MIDAS

10.3 Definitions

Adverse event (AE) An AE is any untoward medical experience or change of an existing condition that occurs during or after treatment, whether or not it is considered to be related to the protocol intervention.

Unexpected Adverse Event [21 CFR 312.32 (a) An AE is unexpected if it is not listed in the investigator's brochure and/or package insert; is not listed at the specificity or severity that has been observed; is not consistent with the risk information described in the protocol and/or consent; is not an expected natural progression of any underlying disease, disorder, condition, or predisposed risk factor of the research participant experiencing the AE.

Expected Adverse Event - Any event that does not meet the criteria for an unexpected event OR is an expected natural progression of any underlying disease, disorder, condition, or predisposed risk factor of the research participant experiencing the adverse event

Serious Adverse Event (SAE) [21 CFR 312.32] is defined as any expected or unexpected adverse event that results in any of the following outcomes:

- Death
- Is life-threatening experience (places the subject at immediate risk of death from the event as it occurred)

- Unplanned hospitalization (equal to or greater than 24 hours) or prolongation of existing hospitalization
- A persistent or significant disability/incapacity
- A congenital anomaly/birth defect
- Secondary Malignancy
- Any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the outcomes listed above (examples of such events include allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasisas of convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse).

Unanticipated problem (UP) Any incident, experience, or outcome that **meets all three** of the following criteria:

1. Unexpected (in term nature, severity, or frequency) given the following: a) the research procedures described in the protocol-related documents such as the IRB approved research protocol, informed consent document or Investigator Brochure (IB); and b) the characteristics of the subject population being studied; **AND**
2. Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcomes may have been caused by the drugs, devices or procedures involved in the research); **AND**
3. Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm) than previously known or recognized.

10.4 Reporting of Unanticipated Problems and Adverse Events

Unanticipated Problems: Most unanticipated problems must be reported to the COH DSMC and IRB **within 5 calendar days** according to definitions and guidelines at http://www.coh.org/hrpp/Pages/hrpp-policies.aspx. Any unanticipated problem that occurs during the study conduct will be reported to the DSMC and IRB by submitting electronically in iRIS (http://iris.coh.org).

Serious Adverse Events - All SAEs occurring during this study, whether observed by the physician, nurse, or reported by the patient, will be reported according to definitions and guidelines at *http://www.coh.org/hrpp/Pages/hrpp-policies.aspx* and **Table 2**. Those SAEs that require expedited reporting will be submitted electronically in iRIS (*http://iris.coh.org/*).

Adverse Events - Adverse events will be monitored by the PMT. Adverse events that do not meet the criteria of serious OR are not unanticipated problems will be reported only in the protocol continuation reports and PMT report (see **Table 2**).

An event determined by the IRB of record to be an Unanticipated Problem (UP) will be communicated to the Investigator and COH DSMC through the COH IRB Operations Director. The DSMC will review the case and make a determination as to whether the study will be suspended, terminated, amended, or allowed to continue without amendment.

Table 2: City of Hope Adverse Event and Unanticipated Problem Reporting Timelines for the DSMC and IRB

Required Reporting Timelines to DSMC for AE/SAEs ***Externally Sponsored Studies***		
Required Reporting Timeframe to DSMC		
Attribution	**UNEXPECTED[1]**	**EXPECTED**
	Death while on active treatment or within 30 days of last day of treatment	
Possibly, Probably, Definitely Unlikely, Unrelated	No DSMC reporting required - IRB reporting may be necessary	
	Death after 30 days of last active treatment/therapy	
Possibly, Probably, Definitely Unlikely, Unrelated	No DSMC reporting required - IRB reporting may be necessary	
	Grades 3 and 4 AND meeting the definition of "serious"	
Possibly, Probably, Definitely Unlikely, Unrelated	No DSMC reporting required - IRB reporting may be necessary	
	Grades 1 and 2	
Possibly, Probably, Definitely	No DSMC reporting required - IRB reporting may be necessary	

Required Reporting Timeframe to IRB of Record		
Attribution	**UNEXPECTED**	**EXPECTED**
	Death	
Possibly, Probably, Definitely	5 calendar days	Annual
Unlikely, Unrelated	Annual	Annual
	Grades 3 and 4 AND meeting the definition of a UP	
Possibly, Probably, Definitely	5 calendar days	Annual
Unlikely, Unrelated	Annual	Annual
	Grade 1 and 2 AND meeting the definition of a UP	
Possibly, Probably, Definitely	5 calendar days	Annual
Unlikely, Unrelated	Annual	Annual

10.5 Additional Reporting

10.5.1 Eisai

We will provide Eisai with copies of all SAEs within 7 working days of occurrence of the SAE. We will report any pregnancy occurring in association with use of an Eisai Product to Eisai.

10.5.2 Novartis

We will provide Novartis with copies of all SAEs within 24 hours of occurrence of the SAE. SAEs will be reported on the MedWatch Form 3500A, which can be accessed at: http://www.accessdata.fda.gov/scripts/MedWatch/MedWatch forms should be sent to the FDA online at the above internet address or at:

MEDWATCH
5600 Fishers Lane
Rockville, MD 20852-9787
Fax: 1-800-FDA-0178 (1-800-332-0178)

Collection of complete information concerning SAEs is extremely important. Full descriptions of each event will be followed by Novartis. Thus, follow-up information which becomes available as the SAE evolves, as well as supporting documentation (e.g., hospital discharge summaries and autopsy reports), should be collected subsequently, if not available at the time of the initial report, and immediately sent using the same procedure as the initial SAE report.

An overdose is defined as the accidental or intentional ingestion of any dose of a product that is considered both excessive and medically important. For reporting purposes, Novartis considers an overdose, regardless of adverse outcome, as an important medical event.

The principal investigator has the obligation to report all serious adverse events to the FDA, IRB, and Novartis Pharmaceuticals Drug Safety and Epidemiology Department (DS&E).
All events reported to the FDA by the investigator are to be filed utilizing the Form FDA 3500A (MedWatch Form).

To ensure patient safety, every SAE, regardless of suspected causality, occurring
• after the patient has provided informed consent and until at least 30 days after the patient has stopped study treatment/participation
• after protocol-specified procedures begin (e.g., placebo run-in, washout period, double-blind treatment, etc.) and 30 days after the patient has stopped study treatment
• after the start of any period in which the study protocol interferes with the standard medical treatment given to a patient (e.g., treatment withdrawal during washout period, change in treatment to a fixed dose of concomitant medication) and until 30 days after the patient has stopped study treatment must be reported to Novartis within 24 hours of learning of its occurrence (fax: 877-778-9739). This includes serious, related, labeled (expected) and serious, related, unlabeled (unexpected) adverse experiences. All deaths

during treatment or within 30 days following completion of active protocol therapy must be reported within 5 working days.

Any SAEs experienced after this 30 days period should only be reported to Novartis if the investigator suspects a causal relationship to the study drug. Recurrent episodes, complications, or progression of the initial SAE must be reported as follow-up to the original episode within 24 hours of the investigator receiving the follow-up information.

A SAE occurring at a different time interval or otherwise considered completely unrelated to a previously reported one should be reported separately as a new event. The end date of the first event must be provided.

The original copy of the SAE Report and the fax confirmation sheet must be kept within the Trial Master File at the study site.

Follow-up information is sent to the same fax number as the original SAE Report Form was sent, using a new fax cover sheet, stating that this is a follow-up to the previously reported SAE, and giving the date of the original report. Each re-occurrence, complication, or progression of the original event should be reported as a follow-up to that event regardless of when it occurs. The follow-up information should describe whether the event has resolved or continues, if and how it was treated, whether the blind was broken or not (if applicable), and whether the patient continued or withdrew from study participation.

If the SAE is not previously documented in the Everolimus Investigator Brochure or Package Insert (new occurrence) and is thought to be related to the Novartis study drug, a DS&E associate may urgently require further information from the investigator for Health Authority reporting. Novartis may need to issue an Investigator Notification (IN), to inform all investigators involved in any study with the same drug that this SAE has been reported.

Suspected Unexpected Serious Adverse Reactions (SUSARs) will be collected and reported to the competent authorities and relevant ethics committees in accordance with Directive 2001/20/EC or as per national regulatory requirements in participating countries.

For Comparator Drugs/Secondary Suspects (Concomitant Medications), all serious adverse experiences will be forwarded to the comapartor drug company by the investigator.

10.4 Pregnancy

Preclinical data regarding reproductive toxicity is described in the most recent Investigator Brochure. The potential reproductive risk for humans is unknown. Women of childbearing potential should be advised to use highly effective contraception methods while they are receiving everolimus and up to 8 weeks after treatment has been stopped.

To ensure patient safety, each pregnancy occurring while the patient is on study treatment must be reported to Novartis within 24 hours of learning of its occurrence. The pregnancy should be followed up to determine outcome, including spontaneous or voluntary termination, details of the birth, and the presence or absence of any birth defects, congenital abnormalities, or maternal and/or newborn complications. The newborn will be followed for at least 12 months.

Pregnancy should be recorded on a Clinical Trial Pregnancy Form and reported by the investigator to the oncology Novartis Drug Safety and Epidemiology Department (DS&E). Pregnancy follow-up should be recorded on the same form and should include an assessment of the possible relationship to the study treatment and any pregnancy outcome. Any SAE experienced during pregnancy must be reported on the SAE Report Form.

10.6 Adverse Event Documentation

All AEs occurring after the subject has signed the informed consent must be fully recorded in the subject's case report form.

Documentation must be supported by an entry in the subject's file. A laboratory test abnormality considered clinically relevant, e.g., causing the subject to withdraw from the study, requiring treatment or causing apparent clinical manifestations, or judged relevant by the investigator, should be reported as an adverse event. Each event should be described in detail along with start and stop dates, severity, relationship to investigational product, action taken and outcome.

10.7 Comprehensive AEs and Potential Risks

The descriptions and grading scales found in the current version of the NCI CTCAE (v.4) will be utilized for AE reporting. A copy of the current CTCAE can be downloaded from the CTEP web site: http://ctep.cancer.gov/reporting/ctc.html. AEs will be collected according to the study calendar schedule in **Section 6.4**.

10.7.1 Eribulin

The most common AEs reported in >1 of 10 subjects receiving eribulin are shown below based on 827 breast cancer subjects who received the recommended dose in Phase II and Phase III breast cancer studies.

AE	Adverse Reactions (all grades)
Blood and Lymphatic System Disorders	
Neutropenia	54.5%
Leucopenia	22.1%
Anemia	20.3%
Nervous system disorders	
Peripheral neuropathy	32%
Headache	11%
Asthenia/Fatigue	52.8%
Mucosal inflammation	9.8%
Pyrexia	16.6%
Gastrointestinal disorders	
Constipation	16.3%
Diarrhea	15.0%
Nausea	35.1%
Vomiting	14.5%
Musculoskeletal and connective tissue disorders	
Arthralgia/Myalgia	12.7%
Back pain	1.9%
Bone pain	1.7%
Pain in extremity	4.7%
Investigations	
Weight decreased	9.7%
Metabolism and nutrition disorders	
Decreased Appetite	18.5%
Respiratory, thoracic, and mediastinal disorders	
Cough	4.7%
Dyspnea	5.6%
Skin and subcutaneous tissue disorders	
Alopecia	49.7%
Infections and Infestations	
Urinary Tract Infection	4.8%

10.7.2 Everolimus

Adverse reactions reported in at least one phase III trial in at least 5% of patients and at a higher rate in the everolimus arm than in the placebo arm are shown below.

System Organ Class	Very common	Common
Infections and infestations	Infections[1]	-
Metabolism and nutrition Disorders	decreased appetite[2]	diabetes mellitus
Vascular disorders		hypertension

Nervous system disorders	dysgeusia, headache	-
Respiratory, thoracic and mediastinal disorders	cough, pneumonitis[3] epistaxis, dyspnea	--
Gastrointestinal disorders	stomatitis[4], diarrhea, nausea, vomiting	dry mouth
Skin and subcutaneous tissue Disorders	rash, dry skin, pruritus nail disorder	Acne
General disorders and administration site conditions	fatigue, asthenia, mucosal inflammation, edema peripheral, pyrexia	-
Investigations	weight decreased	-

[1] Includes all those reported for the system organ class and isolated cases of opportunistic infections, including reactivation of hepatitis B (<1%); [2] Reported as anorexia in C2240 according to MedDRA v11.0; [3] Includes alveolitis, interstitial lung disease, lung infiltration, pneumonitis, pulmonary alveolar hemorrhage, and pulmonary toxicity; [4] Includes aphthous stomatitis and mouth and tongue ulceration

Other notable adverse reactions, occurring in at least one pivotal trial more frequently with everolimus than with placebo, but with an incidence of <5% are listed below. All terms included are based on the highest percentage reported in a pivotal trial.

- *Blood and lymphatic system disorders:* Uncommon: Pure red cell aplasia (<1%).
- *Metabolism and nutrition disorders*: Common: dehydration (2.5%), exacerbation of pre-existing diabetes mellitus (1.1%). Uncommon: new onset of diabetes (<1%).
- *Psychiatric disorders:* Common: insomnia (3.3%).
- *Nervous system disorders*: Uncommon: ageusia (<1%)
- *Vascular disorders*: Common: hemorrhage (4.7%, various locations); Uncommon: deep vein thrombosis (<1%).
- *Cardiac disorders*: Uncommon: congestive cardiac failure (<1%).
- *Respiratory, thoracic and mediastinal disorders*: Common: pulmonary embolism (1.5%), hemoptysis (1.1%); Uncommon: acute respiratory distress syndrome (<1%).
- *Gastrointestinal disorders*: Common: oral pain (3.7%), abdominal pain (3.6%), dyspepsia (2.9%), dysphagia (2.6%).
- *Skin and subcutaneous tissue disorders*: Common: hand-foot syndrome (4.7%), erythema (3.7%).
- *Musculoskeletal and connective tissue disorders*: Common: arthralgia (2.8%).
- *Renal and urinary disorders*: Common: proteinuria (2.5%), renal failure (2.3%, including acute renal failure), increased day-time urination (1.8%).
- *General disorders and administration site conditions*: Common: chest pain (1.1%); Uncommon: impaired wound healing (<1%).

In all phase III trials, the majority of observed key laboratory abnormalities were reported with an incidence of ≥10% (listed in decreasing frequency):

- Decreased hematology parameters include hemoglobin, lymphocytes, platelets, and neutrophils (or collectively as pancytopenia).
- Increased clinical chemistry parameters include cholesterol, triglycerides, glucose, aspartate transaminases, creatinine, alanine transaminases, and bilirubin.
- Decreased clinical chemistry parameters include phosphate and potassium.

Most of observed abnormalities were mild (Grade 1) or moderate (Grade 2). Grade 4 abnormalities include reductions in lymphocytes (2.2%), hemoglobin (2%), and potassium (2%), neutrophils, platelets, and phosphate (each <1%) and increases in creatinine (1%), cholesterol, AST, ALT, bilirubin, and glucose (each <1%).

10.8 Other AE Factors to Consider

Factors to be considered in assessing the relationship of the AE to study drugs include:

- *The Temporal Sequence from Drug Administration*: The event should occur after the drug is given. The length of time from drug exposure to event should be evaluated in the clinical context of the event.
- *Recovery on Discontinuation (de-challenge), Recurrence on Reintroduction (re-challenge)*: Subject's response after drug discontinuation (de-challenge) or subjects response after drug re-introduction (re-challenge) should be considered in the view of the usual clinical course of the event in question.
- *Underlying, Concomitant, Current Diseases*: Each report should be evaluated in the context of the natural history and course of the disease being treated and any other disease the subject may have.
- *Concomitant Medication or Treatment*: The other drugs the subject is taking or the treatment the subject receives should be examined to determine whether any of them may be suspected to cause the event in question.
- *The Pharmacology and Pharmacokinetics of the Test Drug*: The pharmacokinetic properties (absorption, distribution, metabolism and excretion) of the test drug(s), coupled with the individual subject's pharmacodynamics should be considered.

11.0 Statistical Considerations

Primary Objective for Phase I Study

The primary objective of the Phase I portion of the study is to determine the safety and tolerability of everolimus and eribulin, and determine the recommended Phase II dose (RP2D) of the drug combination in patients with resistant metastatic TNBC.

Primary Objective for Phase Ib Study

The primary objective of the Phase Ib portion of the study is to evaluate the event-free survival (EFS) rate for patients with resistant metastatic TNBC at the RP2D of everolimus and eribulin to determine if the drug combination is worthy of further study.

Secondary Objectives for Phase Ib Study

The secondary objectives of the Phase Ib portion of the study are to determine response rate, OS, toxicity, and pharmacokinetics (PK) for everolimus and eribulin in patients with resistant metastatic TNBC.

A further secondary objective is to collect blood, skin punch biopsies, and tumor biopsies before and after treatment from all patients, and perform proteomic analysis to determine the level of inhibition of the PI3K pathway in tumor cells versus non-therapeutic targets.

11.1 Design

11.1.1 Phase I portion

The Phase I portion of this study will use the toxicity equivalence range (TEQR) design of Blanchard and Longmate [35] to evaluate select dose levels of the everolimus in combination with eribulin and to determine the RP2D. In this implementation of the TEQR design, we define the target equivalence range of the DLT rate as 0.20-0.35. DLT rate levels of 0.51 or higher will be considered too toxic and doses that achieve that level will not be revisited. The dose escalation de-escalation rules will be the following. If the rate of participants that experience a DLT is below 0.20 we will escalate, if the rate is above 0.35 we will de-escalate and if it is between 0.20-0.35 we will stay at the current dose. Participants will enter the protocol in cohorts of 3. The starting dose will be dose level 1. This study will end when 12 research participants are studied at a single dose level with a toxicity level below 0.51. Further we also implement the additional rules: 1) terminate the trial if dose level 1 is too toxic, and 2) if the current dose is safe but based on the data the next dose is deemed too toxic, stay at the current dose. The RP2D will be the dose closest to target of 0.25 below 0.51 based on isotonic regression.The dose schedule is provided in Table 11.1 below.

The PMT team for protocol 14036 reviewed the data: dose level 2 was closed after 2 of 3 evaluable participants experienced DLTs an observed rate of 67%; dose level 1, we saw 4 DLTs in 8 evaluable participants giving an observed DLT rate of 50% on this dose. Our equivalence range for DLT rate for this trial is 20-35%, our observed rate of 50% is above 35% so we plan to de-escalate. Two participants experienced grade 4 neutrophil count decrease and 2 experienced mucositis (1 grade 3 DLT, 1 participant received 71% of Everolimus due to grade 2 which is a DLT). Thus, the team decided to amend the protocol to allow for two new dose levels at a lower dose of Eribulin, and start the next cohort of patients at dose level B1.

Table 11.1 Dosing Schedule: cycle length is 21 days

Dose level A	Everolimus (oral)	Eribulin (intravenous)
A-1	2.5mg daily (day 1 to 21)	1.4mg/m^2 on days 1, 8
A1	5mg daily (day 1 to 21)	1.4mg/m^2 on days 1,8
A2	7.5mg daily (day 1 to 21)	1.4mg/m^2 on days 1,8
A3	10mg daily (day 1 to 21)	1.4mg/m^2 on days 1, 8

Dose level B	Everolimus (oral)	Eribulin (intravenous)
B1	5mg daily (day 1 to 21)	1.1mg/m^2 on days 1,8
B-1	2.5mg daily (day 1 to 21)	1.1mg/m^2 on days 1,8

Table 11.2 is a pictorial representation of the dose escalation/de-escalation guidelines for a target toxicity of 25% and ε_1=5% ε_2= 10% giving an equivalence range of 20%-35%. The numbers of research participants treated at the current dose is provided in the columns and the number of research participants experiencing a DLT in the rows. Note that this design is similar to a 3+3 design for the first 3 patients seen at a dose as it escalates if 0 study participants experience DLTs, holds if 1 experiences a DLT (33%), and de-escalates if 2 experience a DLT, differing from a 3+3 at sample sizes. of 6 and above patients as it holds at 33% toxicity and de-escalates at 36% toxicity or above.

Based on the results of 1,000 simulated trials considering 4 toxicity probability schedules (**Table 11.3**) for the 4 doses in combination with the trial specifications listed above, the median sample size was 18 with 95% confidence limits of 12 and 24 and the sample size

Table 11.2: Dose escalation/de-escalation guidelines
(E= escalate, D=de-escalate, S=stay, DU=de-escalate and do not return to this dose).

		Number of research participants treated on the current dose level (Standard sample size based on a cohort size =3 are bolded.)									
		3	4	5	**6**	7	8	**9**	10	11	**12**
Number of Research Participants Experiencing a DLT	0	**E**	E	E	**E**	E	E	**E**	E	E	**E**
	1	**S**	S	S	**E**	E	E	**E**	E	E	**E**
	2	**DU**	D	D	**S**	S	S	**S**	S	E	**E**
	3	**DU**	DU	DU	**D**	D	D	**S**	S	S	**S**
	4				**DU**	DU	D	**D**	D	D	**S**
	5						DU	**DU**	D	D	**D**
	6								DU	DU	**D**
	7										**DU**

at the MTD was ≥ 12 in at least 91% of trials. Note **Table 11.3** provides the rates at which each dose level for each schedule was chosen as the MTD (MTD rate) and the average number of research participants seen at each dose (Ave # Pts.).

Table 11.3: Associated rates each dose level is identified as the MTD and average number of patients studied for 4 dose toxicity probability scenarios

Toxicity Probability Scenarios	Dose levels				
	-1	**1**	**2**	**3**	**No MTD**
Scenario 1	**0.05**	**0.1**	**0.25**	**0.50**	
RP2D rate	**0.03**	**0.29**	**0.56**	**0.12**	**0**
Ave # Pts.	**0.4**	**5.9**	**8.1**	**3.6**	
Scenario 2	**0.025**	**0.05**	**0.20**	**0.40**	
RP2D rate	**0.01**	**0.15**	**0.58**	**0.27**	**0**
Ave # Pts	**0.08**	**4.7**	**8.2**	**5.8**	
Scenario 3	**0.025**	**0.05**	**0.1**	**0.25**	
RP2D rate	**0.01**	**0.05**	**0.27**	**0.66**	**0**
Ave # Pts	**0.12**	**3.8**	**5.6**	**9.1**	
Scenario 4	**0.1**	**0.25**	**0.4**	**0.5**	
RP2D rate	**0.19**	**0.55**	**0.22**	**0.04**	**.01**
Ave #Pts	**2.5**	**8.27**	**4.7**	**1.3**	

11.1.2 Phase Ib portion

In the Phase Ib portion of the trial, a Simon's Optimal two stage design [36] will be used to determine if the combination of everolimus and eribulin results in a greater rate of patients event free at 4 months than eribulin alone. Based on data from the Embrace trial we set the 4 month EFS rate for eribulin to be 45%. We want to determine if the combination has a 4 month EFS rate of 70% or higher, that is to show a 25% improvement. Setting both the type I and type II errors to 0.1 after 12 patients (the number of patients at the RP2D dose at the end of the phase I trial),

- if ≤ 5 patients are event free at 4 months the study will stop.
- If 6 or more patients are event free at 4 months we will add on 15 more patients for a total of 27 at the RP2D.
- If 16 or more patients out of the 27 are event free we will determine the combination worthy of further study.

Primary Endpoints

- Phase I: DLTs as defined in **Section 5.7**, Definition of Dose-Limiting Toxicity.
- Phase Ib: Event free survival (EFS)

Secondary EndpointsProgression Free Survival (PFS)

- Response rate
- Overall survival (OS)
- Toxicity profile
- PK parameters

11.2 Sample Size and Accrual Rate

The expected combined sample size for the Phase I/Ib trial will be 33. We anticipate 18 study participants for Phase I portion, and 15 additional at the RP2D to finish out the Phase Ib portion of the trial (minimum sample size=6 and maximum =45 (Phase I: 24 + Phase Ib: 15 + 6 for unevaluable/ineligible patients over the whole trial)).

At the time of adding schedule B, 14 participants had been treated of which 12 are evaluable for dose escalation. With the addition of 2 more doses we expect to treat 12 to 15 more patients for a total of ~29 on the phase I portion of the trial, thus we expect the trial sample size to stay within the above planned limit of 45.

Our accrual is just over 1 participant a month so the phase I portion should be completed after 2 years from start of the trial. We will need to follow the last patient for 4 months to get EFS at 4 months on the 12 patients studied at the RP2D, and expect to do the initial assessment of activity at approximately 2 1/2 years. If 6 or more of the patients studied at the RP2D are event free we will add on 15 additional patients. Based on the same accrual rate we should complete the accrual of the Phase Ib portion within approximately 12 months or and 42 months after start of the study.

11.3 Statistical Analysis Plan

Tables will be created to summarize all toxicities and side effects by dose, course, organ and severity. Rates and associated 95% confidence limits will be estimated for DLTs at the RP2D, EFS at 4 months and response. Kaplan Meier methods will be used to estimate the median and 95% confidence limits for PFS and OS. Descriptive statistics will be provided for the research participant demographics and PK parameters.

11.4 Definitions

11.4.1 Response and progression

Response and progression will be evaluated every 2 cycles in this study using RECIST Version 1.1. Changes in only the largest diameter (one-dimensional measurement) of the tumor lesions are used in the RECIST Version 1.1 criteria. Note: Lesions are either measurable or non-measurable using the criteria provided below. The term "evaluable" in reference to measurability will not be used because it does not provide additional meaning or accuracy.

Measurable Disease: Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥20 mm with conventional techniques (CT, MRI, x-ray) or as ≥10mm with spiral CT scan. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Non-Measurable Disease: All other lesions (or sites of disease), including small lesions (longest diameter <20mm with conventional techniques or <10mm using spiral CT scan), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonis, inflammatory breast disease, abdominal masses (not followed by CT or MRI), and cystic lesions are all non-measurable.

Target Lesions: All measurable lesions up to a maximum of five lesions per organ and 10 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repeated measurements (either by imaging techniques or clinically). A sum of the longest diameter (LD) for all target lesions will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference by which to characterize the objective tumor response.

Non-Target Lesions: All other lesions (or sites of disease) should be identified as **non-target lesions** and should also be recorded at baseline. Non-target lesions include measurable lesions that exceed the maximum numbers per organ or total of all involved organs as well as non-measurable lesions. Measurements of these lesions are not required but the presence or absence of each should be noted throughout follow-up.

11.4.2 Guidelines for evaluation of measurable disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

Note: Tumor lesions that are situated in a previously irradiated area might or might not be considered measurable. If the investigator thinks it appropriate to include them, the

conditions under which such lesions should be considered must be defined in the protocol.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used to assess the antitumor effect of a treatment.

Clinical Lesions: Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Chest X-Ray: Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

Conventional CT and MRI: These techniques should be performed with cuts of ≤10mm in slice thickness contiguously. Spiral CT should be performed using a 5mm contiguous reconstruction algorithm. This applies to tumors of the chest, abdomen, and pelvis. Head and neck tumors and those of extremities usually require specific protocols.

Ultrasound: When the primary endpoint of the study is objective response evaluation, ultrasound should not be used to measure tumor lesions. It is, however, a possible alternative to clinical measurements of superficial palpable lymph nodes, subcutaneous lesions, and thyroid nodules. Ultrasound might also be useful to confirm the complete disappearance of superficial lesions usually assessed by clinical examination.

Endoscopy and Laparoscopy: The utilization of these techniques for objective tumor evaluation has not yet been fully and widely validated. Their uses in this specific context require sophisticated equipment and a high level of expertise that may only be available in some centers. Therefore, the utilization of such techniques for objective tumor response should be restricted to validation purposes in reference centers. However, such techniques can be useful to confirm complete pathological response when biopsies are obtained.

Tumor Markers: Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response. Specific additional criteria for standardized usage of CA-125 response in support of clinical trials are being developed.

Cytology and Histology: These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (e.g., residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or

stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

11.4.3 Response criteria

11.4.3.1 Evaluation of target lesions

CR: Disappearance of all target lesions.

PR: At least a 30% decrease in the sum of the longest diameter (LD) of target lesions, taking as reference the baseline sum LD.

Progressive Disease (PD): At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions.

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started.

11.4.3.2 Evaluation of non-target lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level.

Incomplete Response/ Stable Disease (SD): Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions.

Although a clear progression of "non-target" lesions only is exceptional, in such circumstances the opinion of the treating physician should prevail, and the progression status should be confirmed at a later time.

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

11.4.3.3 Evaluation of best overall response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

Target Lesions	Non-Target Lesions	New Lesions	Overall Response
CR	CR	No	CR
CR	Incomplete response/SD	No	PR
PR	Non-PD	No	PR

SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having "symptomatic deterioration." Every effort should be made to document the objective progression, even after discontinuation of treatment.

In some circumstances, it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) before confirming the complete response status.

11.4.3.4 Confirmatory measurement/duration of response

Confirmation: To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat assessments that should be performed no less than 4 weeks after the criteria for response are first met. In the case of SD, follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval of 8 weeks.

Duration of Overall Response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.

Duration of Stable Disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started.

11.4.3.5 Response review

All responses will be reviewed by an expert(s) independent of the study at the study's completion. This will include simultaneous review of the patients' files and radiological images.

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12.0 Correlative Translational Studies

12.1 Pharmacokinetics

Patients enrolled in the expanded cohort at the MTD will be asked to undergo serial blood sampling to evaluate the PK of everolimus and eribulin. Although it is unlikely that

these two agents will interact negatively, both drugs are substrates for the membrane transporter P-glycoprotein. Therefore, PK studies will be performed in the expanded cohort treated at the MTD to evaluate the PK of the drug combination.[32-34]

All PK/Plasma Samples will be sent to:

Dr. Tim Synold
Analytical Pharmacology Core Facility (APCF)
Shapiro Bldg., Room 1042
Beckman Research Institute/City of Hope
1500 E. Duarte Rd, Duarte, CA, 91010
Phone (626) 256-4673
Fax – (626) 471-9376
Email – tsynold@coh.org

12.1.1 Everolimus PK sampling (whole blood)

At each of the time points below (**Section 12.3.1**), 5ml of anti-coagulated whole blood will be collected into a purple-top Vacutainer® tube containing EDTA for determination of everolimus. These tubes should be inverted several times to mix. Blood samples should be kept on ice until they can be transferred to the freezer. If you are using plastic Vacutainer ® tubes, the samples can be directly frozen at <-20ºC. If glass tubes are used for collection, whole blood must be transferred to polypropylene vials prior to being frozen at <-20°C. **In either case, plasma should not be separated from whole blood.**

12.1.2 Eribulin PK sampling (plasma)

At each of the time points below (**Section 12.3.1**), 5mL of anti-coagulated whole blood will be collected in green-top Vacutainer ® tubes (sodium or lithium heparin) from a site distal to the site of drug administration at the times shown in the table below. Blood samples will be kept on ice until plasma is separated from whole blood by centrifugation at 1500 x g (within 1 hour). Plasma will be transferred to appropriately labeled polypropylene tubes and stored at <-70°C until analysis. A separate PK flow sheet will be used to record the actual times of blood draws, urine collections and other pertinent information. Each sample will be labeled with the patient's name, medical record number, date of collection, scheduled sample collection time and actual sample collection time.

12.1.3 Sampling Schedule

Everolimus Sample Collection

PK Time Point	Sample Type	Cycle	Day	Collection time 24-hour clock)
WB1	Whole Blood	1	D1 +/- 10 min	00:00
WB2	Whole Blood	1	D1 +/- 10 min	01:00
WB3	Whole Blood	1	D1 +/- 10 min	02:00

WB4	Whole Blood	1	D1 +/- 10 min	04:00
WB5	Whole Blood	1	D1 +/- 10 min	06:00
WB6	Whole Blood	1	D2 +/- 2 hours	24:00
WB7	Whole Blood	2	D1 +/- 10 min	00:00
WB8	Whole Blood	2	D1 +/- 10 min	01:00
WB9	Whole Blood	2	D1 +/- 10 min	02:00
WB10	Whole Blood	2	D1 +/- 10 min	04:00
WB11	Whole Blood	2	D1 +/- 10 min	06:00
WB10	Whole Blood	2	D2 +/- 2 hours	24:00

Eribulin Sample Collection

PK Time Point	Sample Type	Cycle	Day	Sample Description	Collection time 24-hour clock)
P1	Plasma	2	D1	Pre	00:00
P2	Plasma	2	D1	End of infusion	00:05
P3	Plasma	2	D1	10 min	00:10
P4	Plasma	2	D1	15 min	00:15
P5	Plasma	2	D1	30 min	00:30
P6	Plasma	2	D1	1 hr +/- 10 min	01:00
P7	Plasma	2	D1	2 hr +/- 10 min	02:00
P8	Plasma	2	D1	4 hr +/- 10 min	04:00
P9	Plasma	2	D1	6 hr +/- 10 min	06:00
P10	Plasma	2	D2	24 hr +/- 2 hours	24:00
P11	Plasma	2	D3	48 hr +/- 3 hours	48:00
P12	Plasma	2	D4	72 hr +/- 3 hours	72:00
P13	Plasma	2	D8	167 hr +/- 3 hours	167:00

12.1.4 Analytical methods

12.1.4.1 Everolimus blood concentrations

Everolimus blood concentrations will determined in all samples in the Analytical Pharmacology Core Facility (APCF) at the City of Hope using a validated LC-MS/MS method. Briefly, following sample clean-up and LC separation from interfering substances in whole blood, a Micromass Quattro Ultima triple quadruple mass spectrometer operating in positive ion mode is used to detect everolimus according to the mass transition of *m/z* 975.5→908.5 with tacrolimus (*m/z* 821.5→768.5) as the internal standard. The limit of quantitation for everolimus in whole blood is 0.25ng/ml.

12.1.4.2 Eribulin plasma levels

Eribulin plasma levels will be determined using a validated LC/MS/MS analytical assay also available in the Analytical Pharmacology Core Facility (APCF). Briefly, following reversed phase LC separation, eribulin and internal standard (ER-076349) will be monitored by tandem MS with electrospray positive ionization operating at a capillary voltage of 2.9 kV and a cone voltage of 58 V. The detector is set to monitor the parent/daughter transitions [MH+] for E (m/z 730 to m/z 712) and IS (m/z 732 to m/z 681). The lower limit of quantitation of the assay is 0.1ng/ml from a 200 µl aliquot of plasma.

12.1.5 Pharmacokinetic data analysis

Non-compartmental PK analyses of everolimus will be performed using statistical moment theory and according to the rule of linear trapezoids and statistical moment theory. Everolimus PK parameters (Cmax, Ctrough, AUC, CL/F, t1/2) will be determined for each individual and a two-stage approach will be used to describe the study population PK.

Compartmental analyses will be performed for eribulin data using ADAPT II (USC Biomedical Simulations Resource, Los Angeles, CA). Secondary pharmacokinetic parameters (e.g. CLsys, Vd, t1/2's, $AUC_{0-\infty}$) will be determined for each individual and a two-stage approach will be used to describe the study population pharmacokinetics. Population means and standard deviations will be compared to values obtained from patients treated on trials of single agent eribulin.

12.1.6 Specimen collection/documentation

Prior to drug administration on day 1 of cycle 1 of treatment, an indwelling heparin lock should be placed so that serial specimens can be collected. At each sampling time, 1mL of blood will be drawn and discarded to assure that the solution used to maintain catheter patency does not dilute the sample. Even if a patient has a central venous catheter, it is preferable for the day 8 PK samples to be drawn through a peripheral heparin lock. However, if the patient objects or has problems with peripheral venous access, the central venous catheter may be used for PK sampling. In the event that the central venous catheter is used, sufficient blood should be withdrawn before each PK sample to assure that the solution used to maintain catheter patency does not dilute the PK sample. It is important to document whether the sample was collected through a heparin lock or central venous catheter, especially for day 1 sampling.

12.2 Molecular Studies

12.2.1 Blood collection

Blood collection will be performed from patients at the multiple time points for patients enrolled to expansion cohort (RP2D) and consented for additional blood collecton:

- Prior to the first dose of everolimus and eribulin.

- 4 hours, 8 hours after dose of everolimus and eribulin, on day 1, day 4 and day 8 of cycle 1.

A serum specimen for proteomic analysis will consist of 7ml of blood in an uncoated tube (collected in the red top). This procedure is performed under standard sterile medical conditions. The red tube should be kept at cold on wet ice (do not invert) and delivered within 15-30 minutes. Serum will be collected and stored at -80°C for proteomic analysis in Dr John Yim's Laboratory using Luminex and mass spectrometry, with confirming ELISA, such as for VEGF, since everolimus and eribulin are known to influence angiogenesis.

A specimen for peripheral blood mononuclear cells (PBMC) and plasma collection will consist of 10ml of blood drawn into an ACD tube (yellow top). This tube will be kept at room temperature and inverted 5-10 times to prevent coagulation. The tube will be brought to a designated person/lab within 15-30 minutes. The processing and storage of these samples will follow the blood separation protocol established in Dr Yim's laboratory for the purpose of biomarker analyses as above. Lysates will be stored in aliquots at -80°C until analysis.

12.2.2 Skin punch biopsy

Two sets of optional skin punch biopsies (two biopsies per set for a total of four biopsies) will be performed for patients who enrolled to dose expansion cohort (Phase Ib, RP2d) and consented as follows:

- First set
    - Biopsy 1 (3mm diameter) will be performed one week prior to any dosing.
    - Biopsy 2 (3mm diameter) will be performed on the same site as Biopsy 1 on the day of the first dose of eribulin and everolimus; cycle 1 (prior to any dosing).
- Second set
    - Biopsy 3 (3mm diameter) will be performed on a new site on the day of the first dose of eribulin and everolimus; cycle 1 (prior to any dosing).
    - Biopsy 4 (3mm diameter) will be performed on the same site as Biopsy 3, one week later on the day that the second dose of eribulin is given (prior to dosing).

Skin biopsy specimen will be placed in formalin containers and transported to Dr. John Yim's lab for storage. Specimen will be analyzed for several biomarkers in epidermal and vascular tissue (see Section 12.2.4).

12.2.3 Tumor biopsy

Patient will be asked to provide a fresh tumor tissue from a newly obtained core or excisional biopsy of a tumor lesion upon study entry. Newly –obtained is defined as a specimen obtained up to 6 weeks (42 days) prior to initiation of treatment on day 1. Subjects for whom newly-obtained samples cannot be provided (e.g. inaccessible or subject safety concern) may submit an archived specimen only upon agreement from the study PI.

Tumors may be biopsied or resected at time of disease progression and will also be evaluated by proteomic analysis. Tumors for which frozen tissue can be obtained will be evaluated for breast cancer subtype using microarray analysis.

The tumors will be placed in snap frozen in liquid nitrogen and sent to Dr John Yim's laboratory (see **Section 12.2.4**).

Protocol for Formaldehyde-Fixed, Paraffin Embedded Tissue Specimens That May Have Been or Biopsied or Resected Before, During, or After Treatment

Paraffin embedded specimens will be processed in conjunction with the routine duties of the COH Anatomic Pathology Department. Additional collection or storage of research specimens outside of the storage per clinical standard of care (which will include additional tissue biopsy cores or parts of surgical tissue specimens, not required for standard pathological diagnosis) will be allowed and conducted under this protocol.

Protocol Frozen Tissue Specimens That May Have Been or Biopsied or Resected Before, During, or After Treatment

Tissue collection will be performed on patients undergoing clinically indicated biopsies or surgical resection while at COH. Once the tissue is biopsied or surgically removed, the specimen will be taken to the pathology laboratory where the pathologist will ensure that the tissue is adequate for routine pathology analyses (diagnosis, margin status assessment, and other indicated purposes). Then, and only then, if excess tissue remains, samples of that tissue will be harvested for archiving in the tissue bank. This archival tissue will be divided and placed into vials, labeled with the subject's unique identification number (RPN) after all patient identifiers are removed, and flash-frozen in liquid nitrogen. It will then be placed into the City of Hope tissue bank freezer (located in the Department of Pathology/or if necessary due to storage space needs in the Pathology Core-tracked in the same data base/LIMS) at temperatures down to -80°C. This tissue will remain in the freezer for at least a one-month period of time, or longer if needed, to ensure that there is no additional diagnostic pathologic requirement for the frozen specimen. During this time, no analyses will be performed on the specimen. This period of time will be known as the "Fail-Safe" time period. The Fail-Safe time period is intended to allow the pathologists the opportunity to withdraw banked tissue for any additional diagnostic testing they determine is necessary to patient care. After the pathologist determines with final certainty, by the publishing of the official final

pathologic report, that there is no diagnostic pathologic requirement for the frozen specimen, then the archived specimen on that patient will be released for research analyses, under appropriate research protocol approved by the IRB.

Tissue collection for non-diagnostic/research purpose will preferably occur at the time of clinically indicated biopsies in the form of extra biopsy specimens. Alternatively, tissue collection for research purpose can occur under IRB-approved specific research protocols and after the patient signed the appropriate consent form. Patients, who are planning treatment with therapy prior to surgery, or have undergone definitive surgery, or are undergoing biopsy or removal of adjacent tissue or metastatic sites may be asked to consent to have an additional biopsy to collect pre-treatment or post-treatment tumor specimens for banking, or alternatively, allow for access tissue collection, such as lymph nodes, or other tissues (for example, brain metastasis).

These procurements can never compromise the establishment of proper diagnosis and the final say in allowing for such biopsies will reside with the pathologist.

In those patients who consent, the tissue collected at biopsy or in surgery, will be placed fresh frozen into the COH tissue bank freezer (located in the Department of Pathology/or if necessary due to storage space needs in the Pathology Core-tracked in the same data base/LIMS at temperatures down to -80°C. In addition, if samples are available, tissue will be also placed in a labeled plastic vial containing RNAlater™ ("RNAlater tissue protect tubes", Ambien Inc. USA) and subjected to labeling, processing, and banking procedures as described above.

Protocol for Formalin-Preserved Tissue Specimens That May Have Been or Biopsied or Resected Before, During, or After Treatment

If any additional excess tissue remains, or procured during biopsy or surgery, samples of that tissue will be harvested for archiving in a formalin tumor bank in the department of Pathology. This archival tissue will be divided and placed into vials, labeled with the subject's unique identification number (RPN) after all patient identifiers are removed, and tissues will be released for research via IRB-approved protocols.

12.2.4 Proteomic Analysis

All specimens will be processed at Dr. John Yim's lab located at:

John Yim, M.D. Lab
1710 Flower Street, Room 154
Beckman Research Institute/City of Hope
1500 E. Duarte Rd, Duarte, CA, 91010

Analysis of PBMC will be performed by Western blotting for pAKT (Ser473), and AKT, p4E-BP1 (ser65/Thr70) and 4E-BP1, pS6K1 (Thr389) and S6K1, and pS6 (Ser235/236)

and S6. The use of PBMC's for analysis in patients has been published.[1] However, successful demonstration of inhibition in PBMC's can be difficult. Nevertheless, we would prefer to attempt correlative studies in PBMC due to greater acceptance of patients to blood draws rather than skin punch biopsies. It is anticipated that compliance to skin punch biopsies will be lower than collection of PBMC. Furthermore, we are anticipating a synergistic inhibition of the PI3K-Akt-mTOR pathway that may result in more prominent correlative results with the combination of everolimus and eribulin which may improve the success rate in demonstrating inhibition. This should also apply to the skin punch biopsies.

For patients in which pretreatment and post-treatment tumor biopsies were obtained in formalin immunohistochemistry analysis (IHC) will be performed for total AKT and pAKT at Ser473, total 4E-BP1, and p4E-BP1 at Thr70, total S6K1 and pS6K1 at Thr389, total S6, pS6 at Ser235/236 and Ki67. For patients in which fresh frozen tissue are available for both pretreatment and post treatment tumor biopsies, Western blot and IHC will be performed for total AKT and pAKT at Ser473, total 4E-BP1, and p4E-BP1 at Thr70, total S6K1 and pS6K1 at Thr389, total S6, pS6 at Ser235/236 and Ki67.

For patients in which fresh frozen tissue are available for both pretreatment and post treatment tumor biopsies, we will also use the Zeiss PALM Microlaser system for laser capture microdissection of invasive ductal cancer cells only for RNA. The microdissected sample will be catapulted to an adhesive cap and loaded to collection tubes for which total RNA isolation and ultimate microarray analysis will be performed by the Integrated Genomics Core using the Affymetrix Human Genome U133 Plus 2.0 array. Patterns of gene expression will be compared to the established gene expression patterns correlating to 7 MBC subtypes displaying unique gene expression and ontologies.

Skin IHC for pAKT (Ser473), and AKT, p4E-BP1 (ser65/Thr70) and 4E-BP1, pS6K1 (Thr389) and S6K1, pS6 (Ser235/236) and S6 will be performed on 4-uM formalin-fixed paraffin-embedded sections using rabbit polyclonal antibodies (Cell-Signaling Technologies). Immunohistochemical analysis of total AKT and pAKT at Ser473, total 4E-BP1, and p4E-BP1 at Thr70, total S6K1 and pS6K1 at Thr389, total S6, pS6 at Ser235/236 and Ki67 will be performed on 4-μm formalin-fixed paraffin-embedded sections. A histo-score (H-score) will be calculated by counting the percentage of keratinocytes from reparative epidermis or endothelial cells from vascular tissue that are positively stained with low, medium or high intensity. The final score ranging from 0 to 300 and will be calculated by the formula: H-score = (low%) × 1 + (medium%) × 2 + (high%) × 3, where the constants 1, 2 and 3 will be weighting factors for low, medium and high intensity, respectively. Changes between pre and on-therapy expression in paired samples will be calculated as H-score ratio and data analyzed using an analysis of variance one-way test.

13.0 Data Reporting and Protocol Deviations

13.1 Data Reporting

13.1.1 Confidentiality of records

The original data collection forms will be stored in a secure location. Clinical report forms (CRFs) will be stored in the electronic data capture (EDC) system. When results of this study are reported in medical journals or at meetings, identification of those taking part will not be disclosed. Medical records of subjects will be securely maintained in the strictest confidence, according to current legal requirements. They will be made available for review, as required by the FDA, or other authorized users such as the NCI, under the guidelines established by the Federal Privacy Act and rules for the protection of human subjects.

13.1.2 Subject consent form

At the time of registration, the original signed and dated Informed Consent form, HIPAA research authorization form, and the California Experimental Subject's Bill of Rights (for the medical record) and two copies (for the subject and the research record) will be available. All Institutional, NCI, Federal, and State of California requirements will be fulfilled.

Data Collection Forms and Submission Schedule

1.0 All data will be collected using COH data collection forms via an electronic data capture system .

2.0 **ELIGIBILITY CHECKLIST**: The data manager at the registering site will have completed and faxed this form at the time of registration.

3.0 **ON-STUDY FORM (FORM OS)**: Completed on-study forms due within two weeks of registration.

4.0 **TREATMENT FORM (FORM RX)**: Completed treatment forms are due within four weeks of completion of a cycle.

5.0 **ADVERSE EVENT COLLECTION**: Completed adverse events collection form due within four weeks of completion of a cycle.

6.0 **FLOW SHEETS**: Protocol specific flow sheets are to be submitted along with each treatment form.

7.0 **RESPONSE/OFF-STUDY/FOLLOW-UP**: Form F/U is to be submitted each time a patient is evaluated for response and/or new follow-up information is obtained.

8.0 **SUPPLEMENTAL DATA FORM**: The timeline for submission of the

supplemental data form will be protocol specific, if applicable.

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Eligibility Checklist

The Eligibility Checklist must be completed by a protocol nurse or clinical research associate and signed by an authorized investigator prior to registering the subject. See Section 6.5 for the registration procedures.

Prior Therapy Forms and On-Study Forms

Within two weeks of registration, the clinical research associate will submit On Study forms.

13.2 Protocol Deviations

13.2.1 Deviation policy

Brief interruptions and delays may occasionally be required due to travel delays, airport closure, inclement weather, family responsibilities, security alerts, government holidays, etc. This can also extend to complications of disease or unrelated medical illnesses not related to disease progression. These delays will not be considered protocol deviations.

Planned deviations may be permitted in accordance with the COH policy on "Clinical Research Protocol Planned Deviations and Single Subject Exception." These planned deviations, considered "Single Subject Exceptions", are considered an Amendment to the Protocol.

Additionally,

- Missed doses will not be made up.
- If growth factor is needed, it can be administered to patients after cycle 1, but growth factor is not allowed during cycle 1.

13.2.2 Reporting of unplanned deviations

All unplanned deviations will be reported to the COH IRB for review.

13.2.3 Resolving disputes

If there is a dispute among the persons involved in the provision of research treatment, in regard to whether a treatment deviates from the protocol, resolution will be resolved in accordance with the "Clinical Research Protocol Planned Deviations and Single Subject Exceptions" policy.

14.0 Human Subject Issues

14.1 Potential Risks to the Research Subjects

This is a Risk Level 3 study, as defined in the "Guidance, Policy and Procedures for Data and Safety Monitoring for In-House Trials at City of Hope". http://www.infosci.coh.org/prot_office/forms/Guidance.doc. Risks are at least balanced by the potential benefits to subjects and the importance of the knowledge that may result. AEs are expected and a formal plan for monitoring and reporting these AEs is required (outlined in **Section 10**).

14.2 Potential Benefits to Research Subjects and Society

Based on our preclinical findings, we hypothesize that the combination of everolimus and eribulin will have a synergistic effect and will increase clinical activity in patients with TNBC. Previous studies of each of these drugs in MBC show acceptable toxicity, and we hypothesize that this drug combination could offer a novel approach to treat patients with TNBC MBC who are resistant to anthracyclines, taxanes or platinums.

14.3 Alternatives

The alternative is not to participate in the study. Not participating in the study will have no bearing on treatment or follow-up.

14.4 Institutional Review Board

In accordance with COH policies, an IRB that complies with the federal regulations at 45 CFR 46 and 21 CFR 50, 56 and State of California Health and Safety code, Title 17, must review and approve this protocol and the informed consent form prior to initiation of the study. All institutional, NCI, Federal, and State of California regulations must be fulfilled.

14.5 Recruitment of Subjects

All patients will be recruited from patients undergoing cancer treatment at COH.

14.6 Advertisements

Advertisements to include print, media (radio, television, or billboards), telephone scripts, etc., will be reviewed and approved by the IRB prior to their use to recruit potential study subjects.

14.7 Study Location and Performance Sites

This study will be performed at COH.

14.8 Confidentiality

This research will be conducted in compliance with federal and state of California requirements relating to protected health information (PHI). All the medical activity and research will follow HIPPA regulation.

14.9 Financial Obligations and Compensation

There will be no financial obligations incurred by the subject because of participating in this study. All translational studies will be performed solely for research purposes.

If there is a serious medical complication as a result of the research, treatment will be available at COH, but there will be no compensation to the subject for this injury.

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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

IRB #14036

Patient Diary for Everolimus
Everolimus dose: Daily

ASSIGNED DOSE LEVEL: ___________________________________

PATIENT INITIALS: ___________________________________

PATIENT STUDY ID #: ___________________________________

COURSE # : ___________________________________

PATIENT DIARY

Date: Dose taken: Time taken:	Date: Dose taken: Time taken:	Date: Dose taken: Time taken:	Date: Dose taken: Time taken:	Date: Dose taken: Time taken:	Date: Dose taken: Time taken:	Date: Dose taken: Time taken:
Date: Dose taken: Time taken:	Date: Dose taken: Time taken:	Date: Dose taken: Time taken:	Date: Dose taken: Time taken:	Date: Dose taken: Time taken:	Date: Dose taken: Time taken:	Date: Dose taken: Time taken:
Date: Dose taken: Time taken:	Date: Dose taken: Time taken:	Date: Dose taken: Time taken:	Date: Dose taken: Time taken:	Date: Dose taken: Time taken:	Date: Dose taken: Time taken:	Date: Dose taken: Time taken:

***NOTE:* Daily doses should be taken at the same time each day**

Signature of person filling out the diary

Date completed/Returned to Study center

APPENDIX *C*

City of Hope Data Coordinating Center

Registration Procedures

REGISTRATION PROCEDURES FOR PHASE I TRIALS

I. REGISTRATION POLICIES

A. Registrations for Phase I protocols will be done centrally through the City of Hope Data Coordinating Center (COH DCC) in the Division of Clinical Research Information Support (CRIS) between the hours of 8:30 a.m. and 4:30p.m., Monday through Friday (except holidays).

B. Patients must begin protocol therapy within timeframe allowed per protocol in registration section.

C. Pre-study laboratory tests, scans, and x-rays, must be completed prior to registration, within the time frame specified in the protocol (Staging image scans must be performed within 28 days prior to study entray). The eligibility checklist must be completed and signed by the treating physician. Additionally, patients must sign an informed consent and HIPAA authorization form prior to registration.

A patient failing to meet all protocol requirements will not be registered.

II. "HOLDING A SLOT" FOR A POTENTIAL PATIENT

- If a potential patient has been identified for a Phase I trial, the protocol nurse or CRA must contact the Data Coordinating Center (626-256-4673, ext. 64267 or e-mail dcc@coh.org to verify that a dose-level is open.

1. If the current dose-level is open, then the Data Coordinating Center will reserve the "slot" for that patient for 7 days to allow sufficient time to obtain the informed consent. After reserving a slot and consenting the patient, you will have 14 days to work up the patient. (Additional time may be allowed for scheduling of required tests and pending results, but the delay must be cleared by the DCC with the approval of the Protocol PI

before the 14 days expire). Only one reserved slot will be allowed per physician at a new dose level. If no patient is available in that time, the physician that has additional patients for the study will then be given another slot at that dose level. If there are any issues with slot reservations, the matter will be discussed with the Phase I Program Director and the Data Coordinating Center.

a. The protocol nurse or CRA must call back within 14 days days to complete the registration or cancel the patient.

2. If no dose-level is open (based on the criteria for dose expansion or escalation - as described in the protocol), then the Data Coordinator Center can indicate the anticipated date of reopening and a slot may be reserved for that patient on the waiting list.

3. No more than 3 patients may be held on the waiting list for a single protocol.

a. If a patient on the waiting list becomes ineligible, then the protocol nurse or CRA must call to remove the patient from the list in a timely manner.

4. No slot reservation at a given dose level will be made prior to escalation or expansion until the DCC is notified by the Protocol PI or designee of the dose escalation/expansion decision.

II. REGISTRATION PROCEDURES

- Once a patient is eligible, all the pre-study requirements have been fulfilled, and informed consent obtained, then the protocol nurse or CRA must contact the Data Coordinating Center (626-256-4673, ext. 64267 or e-mail dcc@coh.org) and EMAIL a copy of the completed eligibility checklist, required pre-study tests (per protocol – and may include laboratory, CT and pathology reports), signed Informed Consent, signed Patients' Bill of Rights and HIPAA authorization form to dcc@coh.org.

- Upon receipt of the registration documents, the Data Coordinating Center will verify and confirm that the patient is eligible.

- Assign a patient accession number (for example, COH-001, COH-002, etc.).

Register the patient on study centrally (the City of Hope CRA assigned to the trialwill still be responsible for accessioning via MIDAS). If multi-site study, COH DCC staff will be responsible for registering participating site patients at City of Hope.

- Assign the patient to a dose level (as applicable).

Complete and email a Confirmation of Registration form within 24 hours to

Include the COH patient study number and dose level (as applicable) to the study team, which will include the Principal Investigator, treating physician, protocol nurse, CRA and COH IDS Pharmacy.

- Call the protocol nurse and/or CRA to verbally confirm registration.