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DF/HCC Protocol #: 17-355

TITLE: A Pilot Phase 2 Study of Eribulin in Angiosarcoma and Epithelioid hemangioendothelioma (EHE)

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Study agent: Eribulin

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

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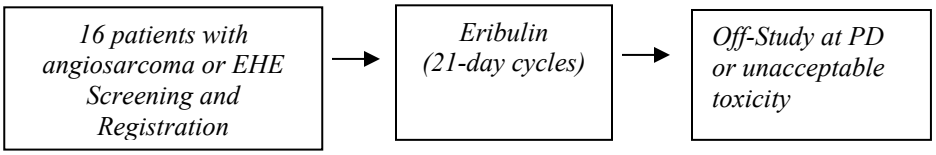
SCHEMA


16 patients with angiosarcoma or EHE Screening and Registration
Eribulin (21-day cycles)
Off-Study at PD or unacceptable toxicity

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1. OBJECTIVES

This is a pilot study to explore the clinical activity of Eribulin Mesylate (HALAVEN, hereafter referred to as Eribulin) as a single agent for the treatment of patients with metastatic or unresectable angiosarcoma or Epithelioid hemangioendothelioma (EHE). We hypothesize that Eribulin will lead to objective responses and/or durable disease control in patients with angiosarcoma or EHE.

1.1 Study Design

We propose a single arm, single stage pilot study to explore the clinical activity of Eribulin in angiosarcoma and EHE with the primary objective of Overall Response Rate (ORR, PR+CR) by RECIST 1.1.

Patients will receive Eribulin on day 1 and day 8 of a 21-day cycle.

Imaging will be obtained every 6 weeks for the first 7 cycles (before C3, C5 and C7) and then every 9 weeks for C7 onwards (C10, C13, etc.).

1.2 Primary Objectives

- To determine the Objective response rate (ORR, partial response [PR]+complete response [CR]) by RECIST 1.1 of angiosarcoma/EHE patients treated with Eribulin

1.3 Secondary Objectives

- To further characterize the clinical activity of Eribulin in this population by:
    - Progression-Free Survival (PFS)
    - Disease Control Rate (DCR = ORR + Stable Disease [SD] at 24 weeks)
- To further characterize the safety profile of Eribulin in this patient population

1.4 Exploratory Objectives

- To determine any correlation between angiosarcoma or sarcoma subtype and response
- To determine any correlation between response and genomic characterization of the sarcoma
- To determine genomic and expression profile changes, via biopsy samples and/or circulating free DNA exome sequencing, over time after treatment with Eribulin

2. BACKGROUND

2.1 Study Disease(s)

The sarcomas are a heterogeneous family of mesenchymal malignancies arising in bone or soft tissue. For patients with metastatic soft tissue sarcoma (STS), single agent or combination chemotherapy has led to a median survival of only 12 months across all histologies.(1, 2) Angiosarcoma (AS) is an uncommon STS that can arise in nearly any organ including the head and neck, breast, extremities, trunk, liver and bone. There is a peak incidence in the 7th decade of life.(3) The cause for most AS is unknown with subsets linked to exposure to radiation, foreign materials, toxins (e.g. vinyl chloride), chronic inflammation or lymphedema (Stewart-Treves syndrome) and genetic syndromes (e.g. BRCA1, NF1, Maffucci's).(4)

Multimodality therapy, including surgery, radiation and standard chemotherapy agents including taxanes and anthracyclines, is the treatment backbone of this disease. There is ongoing research into the role of VEGF-directed therapies and tyrosine kinase inhibitors. However, despite this, angiosarcomas are extremely aggressive tumors with stage for stage worse survival than other STS. For example in one series half of patients had died by 11 months.(5)

Epithelioid hemangioendothelioma (EHE) is a rare vascular sarcoma with a variable clinical course.(3) EHE can develop in virtually any organ and it frequently metastasizes to the lung and liver. Most EHE is typically genomically characterized by the gene fusion WWTR1-CAMTA1. There are no treatments for EHE and where metastatic mortality remains high.

2.2 Study Agent

2.2.1 Eribulin

Eribulin mesylate (HALAVEN®) is a synthetic analog of halichondrin B (HalB), a natural product isolated from the marine sponge Halichondria okadai. HalB is a large polyether macrolide that exerts potent anti-cancer effects in cell-based and animal models of cancer.(6-8) The structurally simplified synthetic analog Eribulin mesylate has shown potent anti-cancer properties in preclinical models.(8)

2.2.2 Therapeutic Pathway

Results of *in vitro* studies demonstrate that eribulin inhibits cell growth with sub- to low-nmol/L half-maximal inhibitory concentration (IC_{50}) values in a wide range of established human cancer cell lines, including breast, colon, prostate, ovarian, small cell and non-small cell lung cancer, as well as histiocytic lymphoma, promyelocytic leukemia, pharyngeal squamous cell carcinoma (head and neck cancer), melanoma, and uterine sarcoma. Eribulin exerts its anti-cancer effects via a tubulin-based antimitotic mechanism, leading to G2/M (GAP 2/mitosis stages of cell cycle)

cell cycle blocks, disruption of mitotic spindles, and ultimately apoptotic cell death after prolonged mitotic blockage.

Eribulin is mechanistically distinct as an inhibitor of microtubule dynamics, leading to inhibition of microtubule growth in the absence of effects on microtubule shortening, and formation of non-productive tubulin aggregates. This pattern of inhibitory effects on microtubule dynamics is not shared by known tubulin-targeted agents.(9)

Eribulin may have additional effects on tumor vascular remodeling and reversal of EMT. It has been reported that the abnormal vasculature present in tumors impairs blood perfusion and oxygenation, leading to hypoxic conditions, which promotes invasion, metastasis, and overall aggressiveness of tumor cells through epithelial mesenchymal transition (EMT)-related processes. Normalization of vascular perfusion is therefore gaining interest as a therapeutic approach to inhibit motility, invasiveness, and aggressiveness of tumor cells.
Preclinical data suggest that eribulin mesylate improves tumor blood perfusion through vascular remodeling, which may indirectly contribute to reduced metastasis, invasion, and reversal of EMT in the tumor microenvironment, leading to a normoxic tumor microenvironment.(10-13)

2.2.3 Clinical Experience with Eribulin

Nine Phase 1 clinical studies have been completed with eribulin. The National Cancer Institute (NCI)-sponsored two Phase 1 studies of eribulin: NCI Study 5730 and NCI Study 7444. Eisai has sponsored seven studies: E7389-A001-101, E7389-A001-102, E7389-E044-103, E7389-J081-105, E7389-E044-108, E7389-E044-109, and E7389-E044-110.

In general, the pharmacokinetics (PK) of eribulin is characterized by a rapid distribution phase, with a prolonged elimination phase after intravenous (IV) infusion. The disposition of eribulin follows linear kinetics over the dose range studied, as shown by consistent dose independent PK parameters ($t_{½}$, clearance [CL], volume of distribution at steady state [V_{ss}]) and similar dose-normalized parameters (C_{max}/Dose, AUC_{0-t}/Dose and $AUC_{0-\infty}$/Dose) between eribulin doses ranging from 0.25 to 1.4 mg/m^2 (E7389-A001-101) and from 0.25 to 4.0 mg/m^2 (E7389-A001-102).

Eribulin demonstrated activity across all identified subgroups of subjects, including subjects with advanced/metastatic breast cancer (MBC) (E7389-A001-201, E7389-J081-221 and E7389-G000-211), advanced/metastatic hormone-refractory prostate cancer (E7389-G000-204), or non-small cell lung cancer (NSCLC) (E7389-A001-202). The response rate, clinical benefit rate (CBR) and PFS were reported across breast cancer phenotypes. Eribulin demonstrated activity in subjects with heavily pretreated MBC who were refractory to anti-cancer therapy, including anthracyclines, taxanes, and/or capecitabine.(14)

Results of an Eisai-sponsored, Phase 2 study conducted by the European Organisation for Research and Treatment of Cancer (EORTC) in advanced STS was reported at the annual conference of the American Society of Clinical Oncology (ASCO) in 2010.(15) These results have since been published.(16) Patients with histological proven advanced STS of high or

intermediate grade with disease progression within 6 months prior to study entry and no more than one prior combination regimen, or two prior single agents for advanced STS, was treated with eribulin 1.4 mg/m² on Days 1 and 8, every 21 days in four STS strata: adipocytic sarcoma (ADI), leiomyosarcoma (LMS), synovial sarcoma, and other types of sarcoma. The primary endpoint was progression-free rate at Week 12 (PFR_{12wks}).

The PFR_{12wks} was 46.9% (95% CI, 29.9 - 62.8) for ADI sarcoma, 44.7% (95% CI, 28.7 - 59.6) for LMS, 31.6% (95% CI, 12.9 - 52.3) for synovial sarcoma and 24% (95% CI, 9.8 - 41.7) in other types of sarcoma.**Error! Reference source not found.**

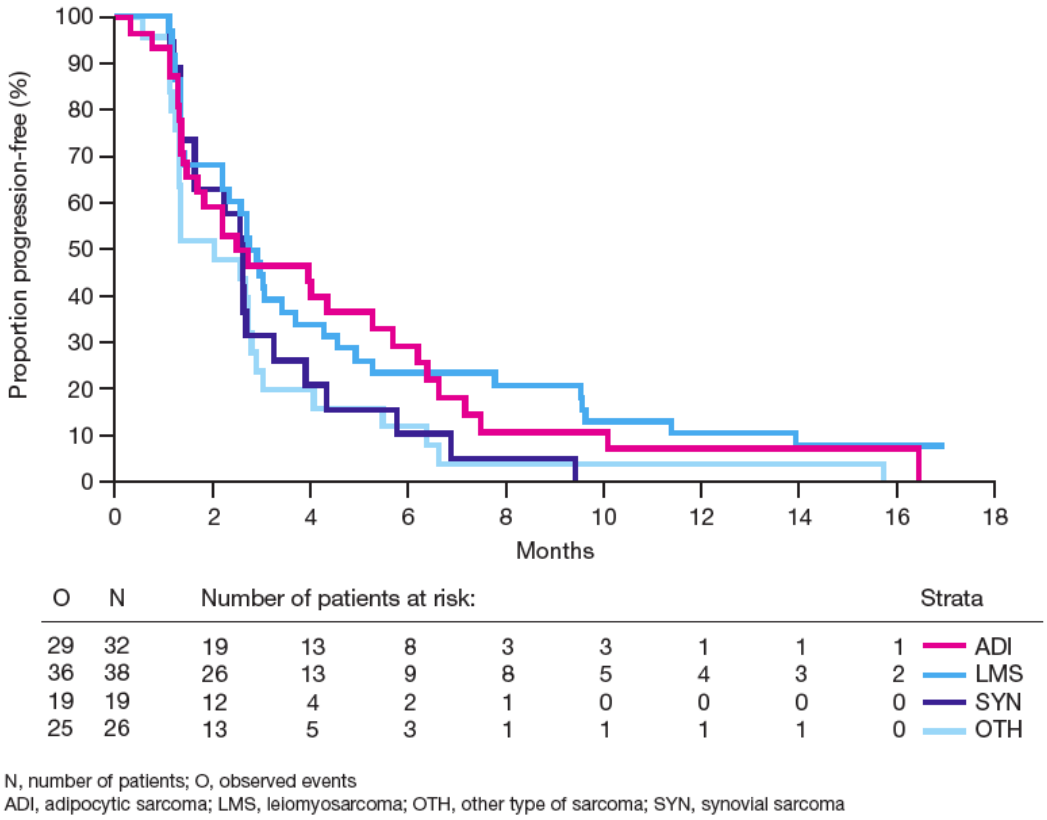
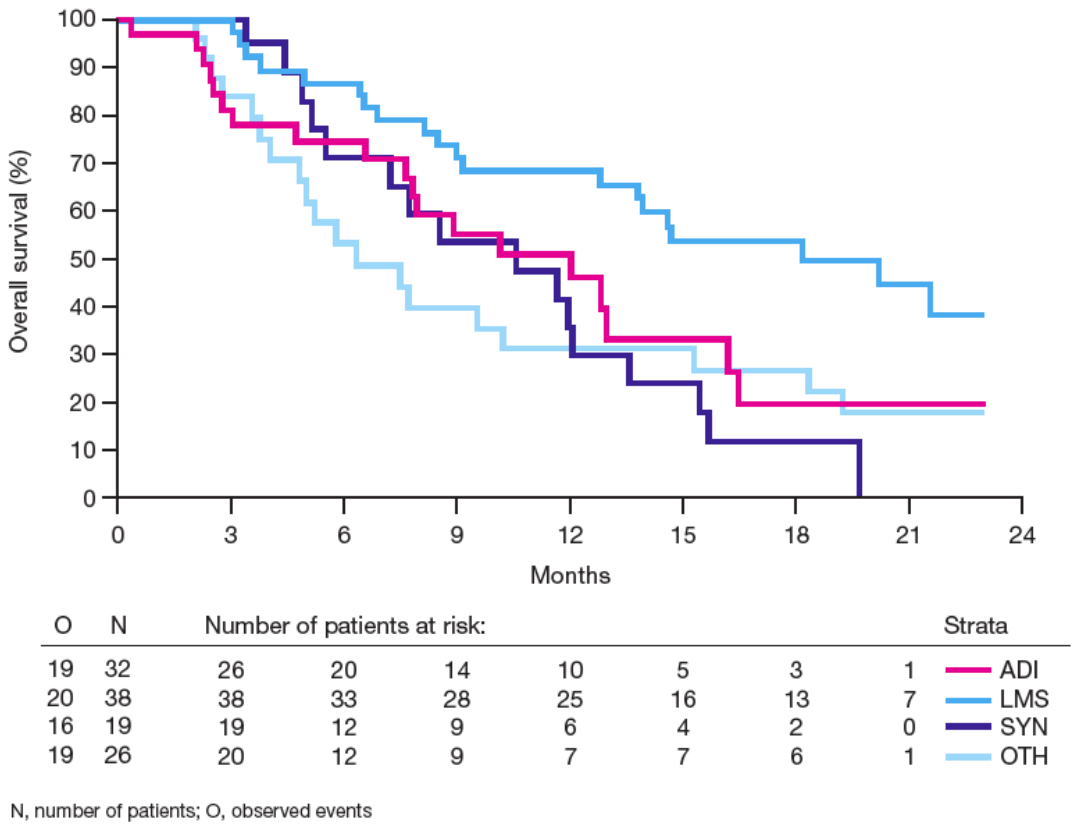


Figure 1 Protocol E7389-E044-207: Proportion Progression-Free

The median PFS for LMS was 2.9 months (95 % CI, 2.4 - 4.6), the OS at 6 months (Figure) from the start of treatment (OS_{6mths}) 86.8% (95% CI, 71.2 - 94.3). The median PFS for ADI sarcoma was 2.6 months (95% CI, 1.7 - 6.2), the OS_{6mths} 74.6% (95% CI, 55.5 - 86.4). The median PFS for synovial sarcoma was 2.6 months (95% CI, 2.3 - 4.3), the OS_{6mths} 71.1% (95% CI, 43.7 - 86.8). The median PFS for other types of sarcoma was 2.1 months (95% CI, 1.4 - 2.9), the OS_{6mths} 52.9% (95% CI, 31.2 - 70.7). Responses included complete response ([CR], one subject in the ADI stratum) and partial responses ([PR], one subject each in ADI, synovial and other type of sarcoma strata).



O	N	Number of patients at risk:							Strata
19	32	26	20	14	10	5	3	1	ADI
20	38	38	33	28	25	16	13	7	LMS
16	19	19	12	9	6	4	2	0	SYN
19	26	20	12	9	7	7	6	1	OTH

N, number of patients; O, observed events

Figure 2 Protocol E7389-E044-207: Overall Survival

The most frequent adverse events (AEs) in the EORTC study were hematologic, with Common Terminology Criteria for Adverse Events (CTCAE) Grade 3 or 4 leukopenia reported in 34.6%, neutropenia reported in 51.9% and anemia reported in 7.1% of subjects, respectively. CTCAE Grade 3 or 4 non-hematologic AEs occurred relatively rarely, with fatigue being the most common (7.1%). CTCAE Grade 3 (and no Grade 4) sensory neuropathy was reported in 3.1% of the subjects. Eribulin was associated with manageable tolerability in subjects with STS.

In addition to these completed clinical studies, a program of two Phase 3 studies in advanced, refractory breast cancer has been undertaken. As of 30 June 2010 enrollment is complete in Study E7389-G000-301 and Study E7389-G000-305.

Study E7389-G000-301 results have since been published. The Phase 3 Eisai Study 301(17) compared eribulin mesylate versus capecitabine monotherapy in patients with locally advanced or metastatic breast cancer. The subject population was predominantly HER2/neu negative (68.5%) and second-line MBC (52%; first- and third-line subjects were also included). Exploratory efficacy subgroupanalysis showed that HER2/neu negative subjects had an increased overall survival (OS) of 2.4 months (median OS of 15.9 months for eribulin mesylate and 13.5 months for capecitabine; nominal P = 0.0299). However, PFS (4 months) and ORR (9.9%) by independent review for eribulin mesylate were similar to the control treatment group (E7389-G000-301 Clinical Study Report).

Study E7389-G000-305 is a Phase 3, open-label, randomized, multicenter study comparing the OS in heavily pretreated subjects with locally recurrent or MBC who have received two to five prior chemotherapeutic regimens (≥2 regimens for advanced disease), including an anthracycline and a taxane (unless contraindicated), when treated with Eribulin or treatment of physician's choice. The primary analysis has been completed and reported at the ASCO conference in 2010 and the results have since been published.(14) The study met its primary endpoint with a statistically significant improvement in OS by a median of 2.5 months in the Eribulin arm. Eribulin demonstrated a manageable tolerability profile in this late-line setting.

Please refer to the Investigator's Brochure for further details.

2.3 Rationale

Clinical data has demonstrated that AS is sensitive to taxanes.(18-22) Indeed at our institution first line therapy is paclitaxel or gemcitabine with taxotere. However, all patients with advanced disease will eventually progress.

Eribulin, a novel microtubule-depolymerizing drug has shown clear activity in metastatic breast and sarcoma patients, despite failure of prior taxanes in many of these patients.

There is now emerging evidence in breast cancer models that Eribulin induces tumor vasculature remodeling as well as altering angiogenesis signaling pathways involved in endothelial cell-pericyte interactions as well as VEGF expression.(12) Moreover, there is further evidence of the Eribulin-mediated vascular remodeling hypothesis in PDX and human breast cancer patients where Eribulin can decreases tumor hypoxia.(23)

In addition to the above there are now anecdotal reports as well as a published report of angiosarcoma patients responding to Eribulin.(24)

Together these data suggest Eribulin's anti-tumor activity is through multiple mechanisms including microtubule targeting, alterations in angiogenesis signaling/vasculature remodeling and tumor hypoxia changes. Thus, we hypothesize that Eribulin will show anti-cancer activity in vascular malignancies including angiosarcoma and progressing epithelioid hemangioendothelioma.

2.4 Correlative Studies Background

In this pilot study, we seek to determine if there are expression and/or genomic variations that correlate with mechanisms of response and/or resistance to Eribulin as a single agent in patients with metastatic or unresectable angiosarcoma. Tumor samples from core needle biopsies pre- and post-treatment will be analyzed by RNA seq and whole exome sequencing. Whole exome sequencing from cell free DNA (cfDNA) will be analyzed from peripheral blood from angiosarcoma patients.

The Broad Institute has sequenced exomes or genomes of over 5000 tumors and has also pursued RNA-seq in hundreds of tumors to discover gene fusions, splice isoforms, and mutations. The Institute has extensive experience in the application of genomics to studies of cancer drug response and resistance in multiple cancer types (Wagle, Emery et al, Journal of Clinical Oncology 2011(25); Van Allen, Wagle, et al, Cancer Discovery, 2014a(26); Wagle, Van Allen, et al, Cancer Discovery, 2014b; Wagle et al, Cancer Discovery, 2014c; Wagle et al, New England Journal of Medicine, 2014(27)). In addition, Broad teams have experience performing sequencing from small amounts of starting material. The Broad Institute now routinely generates WES and RNA-seq data using DNA/RNA obtained from a single core biopsy.

The proposed blood biopsy pilot will be performed in collaboration with Dr. Viktor Adalsteinsson, who leads the Blood Biopsy Team at the Broad Institute. Dr. Adalsteinsson's group consists of a multi-institutional collaboration that is focused on identifying mechanisms of response and resistance to therapies. His group has piloted an approach to performing WES of cfDNA obtained from blood biopsies in patients with metastatic breast cancer (Adalsteinsson et al, In Revision). These pilot efforts have been highly successful in demonstrating their ability to capture and sequence cfDNA. Using an efficient, low-cost method they have developed to assess purity of tumor-derived cfDNA, they found that 85.2% of 251 patients with ER+ MBC had greater than 3% purity of tumor-derived cfDNA and 40.6% had greater than 10%. They performed WES on cfDNA and matched metastatic biopsies from 15 of these patients. WES from cfDNA in these 15 patients identified 90% of the clonal mutations found WES from matched metastatic biopsies (on average; range 60-100%). WES from cfDNA detected 46% of the subclonal mutations identified in the metastatic biopsies (on average; range 10-87%). Copy number alterations were also highly concordant with matched metastatic biopsies, suggesting that cfDNA can also be used for copy number analysis. Dr. Adalsteinsson has also shown that numerous tumor types can be studied by this method (verbal communication), which provides rationale for determining whether this method can be applied to studying cfDNA in angiosarcoma patients.

3. PARTICIPANT SELECTION

3.1 Eligibility Criteria

3.1.1 Metastatic or locally advanced angiosarcoma, treated with at least one prior systemic therapy where no standard of care curable therapy is available OR metastatic or locally advanced malignant and progressive epithelioid hemangioendothelioma (EHE).
3.1.1.1 A maximum of 5 EHE patients will be accrued on this study

3.1.2 Archival tissue confirming the diagnosis must be reviewed by BWH/DFCI/MGH pathology.

3.1.3 Progression on at least one prior systemic therapy or progression during an observation phase of no anti-cancer therapy within the prior 3 months; prior taxanes are allowed

3.1.4 Participants must have measurable disease by RECIST 1.1, defined as at least one lesion that can be accurately measured in at least one dimension (longest diameter to be recorded for non-nodal lesions and short axis for nodal lesions) as ≥20 mm with conventional techniques or as ≥10 mm with spiral CT scan, MRI, or calipers by clinical exam. See Section 11 for the evaluation of measurable disease.

3.1.5 Age ≥ 18 years.

3.1.6 ECOG performance status ≤2 (see Appendix A)

3.1.7 Life expectancy of greater than 3 months

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

– leukocytes	≥3,000/mcL
– absolute neutrophil count	≥1,000/mcL
– platelets	≥100,000/mcL
– total bilirubin	within normal institutional limits
– AST(SGOT)/ALT(SGPT)	≤2.5 × institutional upper limit of normal
– creatinine clearance	≥50 mL/min/1.73 m^2 for participants with creatinine levels above institutional normal.

3.1.9 Baseline QTcF < grade 2

3.1.10 The effects of Eribulin on the developing human fetus are unknown (Please also see exclusion criteria 3.2.6). For this reason and because of the risk of teratogenicity, women of child-bearing potential must agree to use adequate contraception prior to study entry and for the duration of study participation, and 4 months after completion of Eribulin administration. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately. Men treated or enrolled on this protocol must also agree to use adequate contraception prior to the study, for the duration of study participation, and 4 months after completion of Eribulin administration. Please see section 5.6 for complete details on contraception.

3.1.11 Willingness to undergo serial tumor biopsies before and on treatment.

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

3.2 Exclusion Criteria

3.2.1 Participants who have had chemotherapy or radiotherapy within 3 weeks (6 weeks for nitrosoureas or mitomycin C), immunotherapy within 3 weeks, targeted therapies (e.g. small molecule inhibitors such as pazopanib) within 2 weeks prior to entering the study or those who have not recovered from adverse events due to agents administered more than 4 weeks earlier. Not clinically significant or clinically stable adverse events from prior therapy (e.g. immunotherapy related hypothyroidism or insulin-dependent diabetes stable on medication or TKI-related hypertension or rash etc.) is allowed.

3.2.2 Participants who are receiving any other investigational agents.

3.2.3 Participants with with brain or subdural metastases are not eligible, unless they have completed local therapy and have discontinued the use of corticosteroids for this indication for at least 4 weeks before starting treatment in this study. Any signs (e.g. radiologic) or symptoms of brain metastases must be stable for at least 4 weeks before starting study treatment. Participants with leptomeningeal disease should be excluded from this clinical trial because of their poor prognosis and because they often develop progressive neurologic dysfunction that would confound the evaluation of neurologic and other adverse events.

3.2.4 History of allergic reactions attributed to compounds of similar chemical or biologic composition to eribulin.

3.2.5 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure (NYHA Class II), unstable angina pectoris or myocardial infarction within 6 months of enrollment, serious or life-threatening cardiac arrhythmia, subjects with a high probability of Long QT syndrome or QTcF prolongation of $\geq$ 501 mcsec (grade 2) on at least two separate ECG following correction of any electrolyte imbalance or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.6 Females who are breastfeeding or pregnant at Screening or Baseline (as documented by a positive beta-human chorionic gonadotropin [ß-hCG] (or human chorionic gonadotropin [hCG]) test with a minimum sensitivity of 25 IU/L or equivalent units of ß-hCG [or hCG]). A separate baseline assessment is required if a negative screening pregnancy test was obtained more than 72 hours before the first dose of study drug.

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

3.3 Inclusion of Women and Minorities

Both men and women of all races and ethnic groups are eligible for this trial.

4. REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC Institutions

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

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

Following registration, participants may begin protocol therapy. Issues that would cause treatment delays should be discussed with the Overall Principal Investigator (PI). If a participant does not receive protocol therapy following registration, the participant's registration on the study must be canceled. Registration cancellations must be made in OnCore as soon as possible.

4.2 Registration Process for DF/HCC Institutions

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

4.3 General Guidelines for Other Investigative Sites

N/A

4.4 Registration Process for Other Investigative Sites

N/A

5. TREATMENT PLAN

5.1 Treatment Regimen

Eribulin will be administered on day 1 and day 8 of a 21-day cycle. Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 7. Appropriate dose modifications are described in Section 6. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the participant's malignancy.

Regimen Description					
Agent	***Premedications; Precautions***	**Dose**	**Route**	**Schedule**	**Cycle Length**

Eribulin	No premedication requirements	1.4 mg/m^2 administered directly or diluted in NS	IV over 2-5 minutes	Days 1 and 8	21 days

Prophylactic GCSF will be administered according to national guidelines or institution standard.

Body surface area (BSA) will be calculated using the Dubois formula on day 1 of each cycle or per institution standard of care.

5.2 Pre-Treatment Criteria

Prior to any study related testing, patients will sign the informed consent form and undergo medical evaluation to establish their baseline condition and determine eligibility. The following studies will be obtained within 30 days of enrollment for the purpose of baseline assessment:

Complete medical history and physical examination including:

- Complete medical history
- Documentation status of disease
- Documentation of prior therapies
- Documentation of current medications
- Complete physical examination, including vital signs and assessment of ECOG performance status
- Pre-existing conditions will be assessed and evaluated according to the NCI CTCAE v4.03 to establish the patient's baseline condition

Disease-Specific Laboratory Tests- Pathology and Tumor Imaging

- Confirm institutional review of diagnostic pathologic material
- Baseline tumor imaging studies will be either CT or MRI. The modality chosen for any individual patient will be the same throughout the duration of the study.

The following screening tests will be performed within 15 days prior to Day 1 of treatment:

- CBC with differential
- Chemistries: sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, calcium, phosphorus, magnesium, total bilirubin, alkaline phosphatase, AST, ALT, total protein, albumin
- PT and PTT
- Urinalysis
- Urine or serum pregnancy test (for premenopausal women)
- ECG

5.3 Cycle 1, Day 1

Patients who have signed the informed consent form, completed the screening process, and met the criteria for enrollment will be entered into the trial and assigned an identification number. In addition the following will be performed:

- Certification that patient meets all inclusion and exclusion criteria and is able to comply with all requirements of the clinical trial
- Review concomitant medications since screening
- Perform physical examination with vital signs and ECOG performance status
- Laboratory testing – to be done if screening labs were done more than 7 days prior to Day 1. Patient will wait for the lab results on this day to re-confirm lab parameters for eligibility.
    - CBC with differential
    - Chemistries: sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, calcium, phosphorus, magnesium, total bilirubin, alkaline phosphatase, AST, ALT, total protein, albumin
    - Urine or serum pregnancy test (for premenopausal women) if not performed within 7 days of cycle 1 day 1
- Required core or excisional biopsy 1 will occur after registration and prior to cycle 1 day 1.
- It is recommended to correct hypokalemia and hypomagnesemia prior to treatment
- Administer Eribulin cycle 1 day 1 if treatment criteria are met
- HOLD Eribulin administration if:
    - ANC <1000/mm^3
    - Platelets < 75,000/mm^3
- ECG does not need to be repeated if obtained before cycle 1 day 1, unless clinically indicated

5.4 Cycle 1 day 8, Subsequent cycles day 1 and day 8

- See section 6 for Criteria for Treatment Continuation and dosing delays/modifications
- Review concomitant medications
- Perform physical examination with vital signs (temperature, heart rate, blood pressure, oxygen saturation, respiratory rate, and weight) and ECOG performance status
- Laboratory testing
    - CBC with differential
    - Chemistries: sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, calcium, phosphorus, magnesium, total bilirubin, alkaline phosphatase, AST, ALT, total protein, albumin
- ECG for QTc on day 1 of each cycle for cycles 2 and 3. See section 6.3. ECG monitoring on day 1 should be continued for patients with bradyarrhythmias, patients on drugs known to prolong the QT, and patients with electrolyte abnormalities. If there is no QT prolongation for cycles 2 and 3, ECG monitoring on day 1 may be discontinued.
- Administer Eribulin if treatment criteria are met
- It is recommended to correct hypokalemia and hypomagnesemia to grade 1 or better prior to treatment

- HOLD Eribulin administration if:
  - ANC <1000/mm^3
  - Platelets < 75,000/mm^3
  - CTCAE Grade 3 or 4 AE attributable to Eribulin with the specific exclusion of Grade 3 or 4 lymphopenia or Grade 3 hypophosphatemia, hypokalemia, hypocalcemia or hypomagnesemia responsive to oral supplementation

5.5 Eribulin Administration

Eribulin will be administered of 1.4 mg/m2 as an IV infusion over 2-5 minutes on Days 1 and 8 of every 21-day cycle.

Before dose administration, the amount of Eribulin mesylate needed for each subject must be calculated in the following manner:
1. Scheduled dose (mg/m^2) × body surface area (m^2) = Dose (mg)
2. Dose (mg) × 2 = the number of mL of Eribulin mesylate to withdraw from vials for administration.

Body surface area (BSA) will be calculated using the Dubois formula on day 1 of each cycle or per institution standard of care.

Height and body weight will be recorded during the Screening Period. Thereafter, body weight will be recorded on Day 1 of each treatment cycle to recalculate BSA (in the event that weight has changed by 5% or more).

5.6 General Concomitant Medication and Supportive Care Guidelines

Any medications, with the exceptions noted below, which are considered necessary for the patient's welfare, and which are not known to interact with the study medication, may be given at the discretion of the Investigator, providing the medications, the doses, dates and reasons for administration are recorded in the eCRF.

In addition, any unplanned diagnostic, therapeutic or surgical procedure performed during the study period must be recorded in the eCRF.

All medications (prescriptions or over-the-counter medications) continued at the start of the trial or started during the study or until 30 days from the end of the last protocol treatment and different from the study medication must be documented.

Contraception:
Patients of child bearing potential and their partners, who are sexually active, must agree to the use of two highly effective forms of contraception throughout their participation in the study and for 4 months after last dose of study drug(s).

- Condom with spermicide and one of the following:
- Oral contraceptive or hormonal therapy (e.g. hormone implants)

- Placement of an intra-uterine device

Acceptable non-hormonal birth control methods include:

- Total sexual abstinence. Abstinence must be for the total duration of the study and the drug washout period.
- Vasectomised sexual partner plus male condom. With participant assurance that partner received post-vasectomy confirmation of azoospermia
- Tubal occlusion plus male condom with spermicide
- Intrauterine Device (IUD) plus male condom+spermicide. Provided coils are copper-banded

Acceptable hormonal methods:

- Etonogestrel implants (eg, Implanon, Norplan)+male condom with spermicide
- Normal and low dose combined oral pills+male condom with spermicide
- Norelgestromin/ethinyl estradiol (EE) transdermal system+male condom with spermicide
- Intravaginal device+male condom with spermicide (eg, EE and etonogestrel)
- Cerazette (desogestrel)+male condom with spermicide. Cerazette is currently the only highly efficacious progesterone based pill.

Growth factor is permitted according to institution and national guidelines.

No drug-drug interactions are expected with cytochrome P-450 (CYP) 3A4 inhibitors and P-glycoprotein (P-gp) inhibitors. The effect of ketoconazole, a strong inhibitor of CYP3A4 and a P-gp inhibitor, on the PK of eribulin was studied in an open-label, two-treatment, two-sequence, two-way crossover trial in 12 subjects with advanced solid tumors. The mean dose-normalized AUC values were similar when eribulin was administered with or without ketoconazole (ratio of the mean AUC: 0.97; 90% CI, 0.83 - 1.12). Population PK analysis showed no effect of CYP3A4 inducers on eribulin exposure. Eribulin does not inhibit CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1 or CYP3A4 enzymes or induce CYP1A2, CYP2C9, CYP2C19 or CYP3A4 enzymes at relevant clinical concentrations. Eribulin is not expected to alter the plasma concentrations of drugs that are substrates of these enzymes.

Palliative Radiation:

Patients are permitted to receive palliative radiation at any point provided the radiated lesion is not a RECIST 1.1 target. Eribulin should be held during radiation.

5.7 Criteria for Taking a Participant Off Protocol Therapy

Duration of therapy will depend on individual response, evidence of disease progression and tolerance. In the absence of treatment delays due to adverse event(s), treatment may continue until one of the following criteria applies:

- Disease progression
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s)

- Participant demonstrates an inability or unwillingness to comply with the medication regimen and/or documentation requirements
- Participant decides to withdraw from the protocol therapy
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the judgment of the treating investigator

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

An ODQ Treatment Ended/Off Study Form will be filled out when a participant is removed from protocol therapy. This form can be found on the DF/HCC website at http://www.dfhcc.harvard.edu/research/clinical-research-support/document-library-forms-sops-etc/.

In the event of unusual or life-threatening complications, treating investigators must immediately notify the Overall PI, Gregory M. Cote MD PhD at 617-724-4000 or pager 12865.

5.8 Duration of Follow Up

End of treatment assessment for all subjects will be collected within 28 days of last dose. All AEs must be followed for 28 days after the subjects last dose, or until resolution, whichever comes first. All SAEs must be followed to resolution or, if resolution is unlikely, to stabilization.

5.9 Criteria for Taking a Participant Off Study

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

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

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

For Decentralized Subject Registrations, the research team updates the relevant Off Treatment/Off Study information in OnCore.

6. DOSING DELAYS/DOSE MODIFICATIONS

Dose delays and modifications will be made as indicated in the following table(s). The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 will be utilized for dose delays and dose modifications. A copy of the CTCAE version 4.03 can be downloaded from the CTEP website http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

6.1 Eribulin Dosing Delays

- On day 1 or day 8 HOLD Eribulin administration if:
  - ANC $\leq$1000/mm^3
  - Platelets $\leq$ 75,000/mm^3
  - CTCAE Grade 3 or 4 AE attributable to Eribulin with the specific exclusion of Grade 3 or 4 lymphopenia or Grade 3 hypophosphatemia, hypokalemia, hypocalcemia, or hypomagnesemia responsive to oral supplementation
- Day 1: If Eribulin cannot be administered on Day 1, the dose should be delayed until recovery to above these values. The Day 1 dose will be rescheduled for when the criteria for Eribulin administration are met. The dose of Eribulin must be reduced following a dose delay in accordance to the instructions for dose reduction.
- Day 8: If Eribulin cannot be administered on Day 8, the dose should be delayed until recovery to above these values. The Day 8 dose will be delayed for a maximum of 7 days
  - If the criteria above for Eribulin administration are met on, or before Day 15 of a cycle, administer Eribulin at a reduced dose according to section 6.2
  - Eribulin administration on Day 1 of the next cycle must be NO SOONER than 14 days later.
  - If the criteria above for Eribulin administration are NOT being met by Day 15 of a cycle, OMIT the Day 8 dose of Eribulin for that cycle and administer Eribulin at a reduced dose on Day 1 of the next scheduled cycle according to section 6.2.
- Clinical judgment will be used to determine appropriate management of the patient during any adverse event. Temporary interruption or permanent discontinuation of the study drug should be considered if clinically indicated.
- As long as the patient is deriving clinical benefit, there is no limit to the length of time Eribulin may be held.

6.2 Instructions for Dose Reduction

- Do not re-escalate Eribulin dose after it has been reduced.
- Reduce the dose of Eribulin if any of the following values are present after the preceding dose:
  - Absolute Neutrophil Count <1,000/mm^3 with fever and/or infection, or <500/mm^3 for more than 7 days, despite use of growth factors
  - Platelet Count <50,000/mm^3 requiring platelet transfusion, or <25,000/mm^3
  - Possibly, probably or definitely related non-hematologic, clinically significant toxicity CTCAE Grade 3 or 4
  - Day 8 dose delay or omission due to treatment-related toxicity other than outlined above under neutropenia
  - Recurrence of any Grade 3 or 4 event despite reduction to 0.7 mg/m^2, consider discontinuation

Dose Level	Eribulin Dose
Dose 1	1.4 mg/m^2
Dose -1	1.1 mg/m^2

Dose -2	0.7 mg/m^2

6.3 Instructions for QTc Interval Prolongation

- QTc prolongation has been observed in patients receiving Eribulin. ECG monitoring is required for screening, and day 1 for cycle 2 and 3. It is recommended to continue day 1 monitoring beyond cycle 3 for subjects during the treatment who have:
  - Grade 2 QTc interval prolongation
  - Clinically relevant electrolyte abnormalities
  - Bradyarrhythmias and/or congestive heart failure
  - Coadministration of medicinal products known to prolong the QT interval, including Class Ia and III anti-arrhythmics
- For subjects who develop Grade 3 or Grade 4 QTc interval prolongation, discontinue Eribulin and monitor ECGs and electrolytes frequently until the QTc interval returns to baseline. If a reversible etiology not related to Eribulin is determined to be the cause of the QTc interval prolongation rechallenge may be considered after discussion with the overall PI and resolution to grade 2 or better QTc interval prolongation.

6.4 Instructions for Peripheral Neuropathy

Grade 3 and 4 peripheral neuropathy has been observed in patients receiving Eribulin. Patients should be monitored for signs of neuropathy. For patients with grade 3 or 4 peripheral neuropathy Eribulin should be held until improvement to Grade 2 or better. Dose reductions apply as per section 6.2.

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

7.1 Definitions

7.1.1 Adverse Event (AE)

An adverse event (AE) is any untoward medical occurrence in a patient or clinical investigation subject administered an investigational product. An AE does not necessarily have a causal relationship with the medicinal product.

All such AEs will be collected regardless of expectedness or assumed relationship to study drug.

The criteria for identifying AEs in this study are:

- Any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of an investigational product, whether or not considered related to the investigational product (Note: Every sign or symptom should not be listed as a separate AE if the applicable disease [diagnosis] is being reported as an AE)
- Any new disease or exacerbation of an existing disease. However, worsening of the primary disease should be captured under efficacy assessments as disease progression rather than as an AE.

- Any deterioration in nonprotocol-required measurements of a laboratory value or other clinical test (eg, ECG or x-ray) that results in symptoms, a change in treatment, or discontinuation of study drug
- Recurrence of an intermittent medical condition (eg, headache) not present pretreatment (Baseline)
- An abnormal laboratory test result should be considered an AE if the identified laboratory abnormality leads to any type of intervention, withdrawal of study drug, or withholding of study drug, whether prescribed in the protocol or not

All AEs, regardless of relationship to study drug or procedure, should be recorded beginning from the time the subject signs the study ICF through the last visit. SAEs will be collected for 28 days after the last dose.

Abnormal laboratory values should not be listed as separate AEs if they are considered to be part of the clinical syndrome that is being reported as an AE. It is the responsibility of the investigator to review all laboratory findings in all subjects and determine if they constitute an AE. Medical and scientific judgment should be exercised in deciding whether an isolated laboratory abnormality should be classified as an AE. Any laboratory abnormality considered to constitute an AE should be reported on the Adverse Event CRF.

Abnormal ECG (QTcF) results, if not otherwise considered part of a clinical symptom that is being reported as an AE, should be considered an AE if the QTcF interval is more than 450 ms and there is an increase of more than 60 ms from baseline. Any ECG abnormality that the investigator considers as an AE should be reported as such.

All AEs must be followed for 28 days after the subject's last dose, or until resolution, whichever comes first. All SAEs must be followed to resolution or, if resolution is unlikely, to stabilization.

Subjects with onset of an AE or deterioration of a preexisting AE will be followed until resolution to baseline, start of a new anticancer treatment, or death.

Every effort must be made by the investigator to categorize each AE according to its severity and its relationship to the study treatment.

Assessing Severity of Adverse Events

Adverse events will be graded on a 5-point scale according to Common Terminology Criteria for Adverse Event (CTCAE v4.03) Investigators will report CTCAE grades for all AEs (for both increasing and decreasing severity).

7.1.2 Serious Adverse Event (SAE)

A serious adverse event (SAE) is any untoward medical occurrence that at any dose:

- Results in death

- Is life-threatening (ie, the subject was at immediate risk of death from the adverse event as it occurred; this does not include an event that, had it occurred in a more severe form or was allowed to continue, might have caused death)
- Requires inpatient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect (in the child of a subject who was exposed to the study drug)

Other important medical events that may not be immediately life-threatening or result in death or hospitalization but, when based on appropriate medical judgment, may jeopardize the subject or may require intervention to prevent one of the outcomes in the definition of SAE listed above should also be considered SAEs. Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in such situations.

In addition to the above, events associated with special situations include pregnancy or exposure to study drug through breastfeeding; AEs associated with study drug overdose, misuse, abuse, or medication error. These events associated with special situations are to be captured using the SAE procedures but are to be considered as SAEs only if they meet one of the above criteria. All AEs associated with special situations are to be reported on the CRF whether or not they meet the criteria for SAEs.

All SAEs must be followed to resolution or, if resolution is unlikely, to stabilization.

7.1.3 Death

Death as such is the outcome of a SAE and should not be used as the SAE term itself. The cause of death should be recorded as the SAE term instead. When available, the autopsy report will be provided to the Sponsor.
Grade 5 should be used for events which lead immediately and directly to death, and grade 4 should be used with outcome death for events which lead to death after a longer time period, and that may also be linked to additional morbidities.

7.1.4 Life-threatening Event

Any event in which the patient was at risk of death at the time of the event is considered life-threatening; it does not refer to an event which hypothetically might have caused death if it were more severe.

7.1.5 Hospitalization or Prolongation of Hospitalization

Any AE requiring hospitalization (or prolongation of hospitalization) that occurs or worsens during the course of a patient's participation in a clinical trial must be reported as a SAE unless exempted from SAE reporting. Prolongation of hospitalization is defined as any extension of an inpatient hospitalization beyond the stay anticipated/required for the initial admission, as determined by the Investigator or treating physician.

The following hospitalizations are not considered to be SAEs because there is no "adverse event" (ie, there is no untoward medical occurrence) associated with the hospitalization:

- Hospitalizations for respite care
- Planned hospitalizations required by the protocol
- Hospitalization planned before informed consent (where the condition requiring the hospitalization has not changed after study drug administration)
- Hospitalization for administration of study drug or insertion of access for administration of study drug
- Hospitalization for routine maintenance of a device (eg, battery replacement) that was in place before study entry

Other situations that MUST NOT be considered as hospitalizations are the following:

a. An emergency visit due to an accident where the patient is treated and discharged.
b. When the patient is held 24 hours for observation and finally is not admitted.
c. Planned treatments at sites not associated to a hospital and generally considered as minor surgical procedures (i.e., laser eye surgery, arthroscopy, etc.).

7.1.6 Unlisted/Unexpected Adverse Event

An AE, the nature or severity of which is not consistent with the applicable reference safety information.
The Sponsor and the Sponsor designee will use as the reference safety information for the evaluation of listedness/expectedness in the IB and the Summary of Product Characteristics (SmPC) for Eribulin as described in the FDA package insert.

7.1.7 Adverse Reactions

All untoward and unintended responses to an investigational medicinal product related to any dose administered. This definition covers also medication errors and uses outside what is foreseen in the protocol, including overdose, lack of efficacy, misuse and abuse of the product.

Overdose	Accidental or intentional use of the study drug in an amount higher than the protocol-defined dose *(If applicable, define the study-specific criteria for overdose that should be applied when determining whether an overdose occurred.)*
Misuse	Intentional and inappropriate use of study drug not in accordance with the protocol *(Use the following definition, if appropriate, for postmarketing studies)* Intentional and inappropriate use of study drug not in accordance with the prescribed or authorized dose, route of administration, or indication(s) or use of prescription-only study drug without a prescription
Abuse	Sporadic or persistent intentional excessive use of study drug accompanied by harmful physical or psychological effects

Medication error	Any unintentional event that causes or leads to inappropriate study drug use or subject harm while the study drug is in the control of site personnel or the subject.

All AEs associated with overdose, misuse, abuse, or medication error should be captured on the Adverse Event CRF and also reported using the procedures detailed in Reporting of Serious Adverse Events (Section 7.2.2) even if the AEs do not meet serious criteria. Abuse is always to be captured as an AE. If the AE associated with an overdose, misuse, abuse, or medication error does not meet serious criteria, it must still be reported using the SAE form and in an expedited manner but should be noted as nonserious on the SAE form and the Adverse Event CRF.

7.1.8 Adverse Events Related to the Study Drug

An AE is considered related to a study drug/IMP if the Investigator's assessment of causal relationship to the IMP(s) is "Y (yes)" (see Section 7.1.10).

The Investigator will assess the causal relationship of the IMP(s) to the SAE.

The Sponsor may also consider related to the study drug(s)/IMP(s) those events for which the Investigator assesses the causal relationship with the IMP(s) as "Uk (unknown)" when it cannot rule out a role of the IMP(s) in the event.

7.1.9 Expedited Reporting

The Sponsor is responsible for the appropriate expedited reporting according to the applicable legislation.

7.1.10 Assessment of Causal Relationship to the Study Drug

Assessing Relationship to Study Treatment

Items to be considered when assessing the relationship of an AE to the study treatment are:

- Temporal relationship of the onset of the event to the initiation of the study treatment
- The course of the event, especially the effect of discontinuation of study treatment or reintroduction of study treatment, as applicable
- Whether the event is known to be associated with the study treatment or with other similar treatments
- The presence of risk factors in the study subject known to increase the occurrence of the event
- The presence of nonstudy, treatment-related factors that are known to be associated with the occurrence of the event

Classification of Causality

The relationship of each AE to the study drug will be recorded on the CRF in response to the following question:

Is there a reasonable possibility that the study drug caused the AE?

Yes (related) A causal relationship between the study drug and the AE is a reasonable possibility.

No (not related) A causal relationship between the study drug and the AE is not a reasonable possibility.

7.2 Adverse Events Reporting Procedures

7.2.1 Reporting Adverse Events

The Sponsor will collect AEs until 28 days after administration of the last dose of study drug(s)/IMP(s), or until resolution, whichever comes first. All AEs suspected to be related to the study drug/IMP must be followed-up after the time of therapy discontinuation until the event or its sequelae resolve or stabilize at an acceptable level to the Investigator and the Sponsor.

All AEs, including misuse, overdose and abuse, must be recorded in English using medical terminology in the source document and the CRF. Whenever possible, the Investigator will record the main diagnosis instead of the signs and symptoms normally included in the diagnoses.

Investigators must assess severity (grade) of the event following the NCI-CTCAE v. 4 and assign a relationship to each study drug(s)/IMP(s); and pursue and obtain information adequate both to determine the outcome and to assess whether it meets the criteria for classification as a SAE requiring immediate notification to the Sponsor and the Sponsor designee. The Investigator must provide any relevant information as requested by the Sponsor or the Sponsor designee in addition to that on the CRF.

Abnormal laboratory tests occurring during the study should only be recorded in the AE section of the CRF if the disorder:

- Is associated with clinically significant symptoms, and/or
- Leads to a change in study dosing or discontinuation from the study, significant additional concomitant drug treatment or other therapy, and/or
- Leads to any of the outcomes included in the definition of a SAE.

Otherwise laboratory results should be reported in the corresponding section of the CRF (e.g. biochemistry, hematology).

All episodes of febrile neutropenia must always be reported within 24 hours following the same procedure for reporting SAEs (see Section 7.2.2), including episodes that occurred in patients without seriousness criteria. For these cases, the seriousness criterion should be reported as a medically significant event.

7.2.2 Reporting Serious Adverse Events

The Sponsor will collect SAEs from the time of signing of the informed consent form (ICF) until 28 days after administration of the last dose of study drug(s)/IMP(s) or until the start of a new

antitumor therapy or until the date of death, whichever occurs first. Beyond this period of time, only those SAEs suspected to be related to the IMP will be collected. Nonetheless, the Sponsor will evaluate any safety information that is spontaneously reported by an Investigator beyond the time frame specified in the protocol.

All SAEs (as defined above) occurred after patient registration regardless of relationship to the study drug(s)/IMP(s) must be reported immediately and always within 24 hours to the Sponsor and the Sponsor designee: electronically by completing the applicable e-CRF section.

SAEs will be reported within one business day of notification to the Sponsor Dr. Gregory Cote by email (gcote @mgh.harvard.edu) or telephone (617-726-8748) and Eisai Safety Center Safety Hotline (ESI_Safety@eisai.com or Right Fax: 1-732-791-1111) using Eisai SAE/AEASS report form.

SAEs occurring during the screening phase (e.g., from ICF signature to randomization), SAEs that may occur off-study, or in case the electronic system fails or is not available. SAEs initially reported by alternative methods (not electronically), must be followed by a completed electronic SAE reporting on e-CRF from the investigational staff within one business day.

All SAEs suspected to be related to the IMP(s) must be followed until the event or its sequelae resolves or stabilizes at an acceptable level by the Investigator.

7.2.3 Reporting Pregnancy Cases Occurred within the Clinical Trial

National regulations require that clinical trial Sponsors collect information on pregnancies occurring during clinical trials, in which exposure to the IMP(s) at any time during pregnancy, via either maternal or paternal exposure, is suspected.

Therefore, pregnancy and suspected pregnancy (including a positive pregnancy test regardless of age or disease state) of patient occurring while the patient is on study drug, or within 28 days after the administration of the last dose of the study drug(s)/IMP(s), are considered immediately reportable events. Beyond this timeframe, the investigator will report any pregnancy if there is any suspicion that the study drug(s)/IMP(s) might have an impact on the occurrence of the pregnancy.

The Investigator will report the following events immediately and always within 24 hours from first knowledge:

- Any occurrence of a pregnancy where any kind of exposure to the IMP(s) is suspected.
- Possible exposure of a pregnant woman.
- All reports of elevated/questionable or indeterminate beta human chorionic gonadotropins (β-hCGs).

Immediately after detecting a case of suspected pregnancy in a patient, the decision on her continued participation in the clinical trial will be jointly taken by the patient, the Investigator and the Sponsor, with the patient's best interest in mind. A decision to continue the pregnancy will require immediate withdrawal from the trial.

Any pregnancy, suspected pregnancy, or positive pregnancy test must be reported to the Sponsor and the Sponsor designee immediately using the Pregnancy Report form.

The Investigator will follow the pregnancy until its outcome, and must notify the Sponsor and the Sponsor designee the outcome of the pregnancy within 24 hours of first knowledge as a follow-up to the initial report.

For any event during the pregnancy which meets a seriousness criterion (including fetal or neonatal death or congenital anomaly) the Investigator will also follow the procedures for reporting SAEs (complete and send the SAE form to the Sponsor and the Sponsor designee within 24 hours of the Investigator's knowledge of the event).

All neonatal deaths that occur within 30 days of birth should be reported, without regard to causality, as SAEs. In addition, any infant death at any time thereafter that the Investigator suspects is related to the exposure to the study drug(s)/IMP(s) should also be reported to the Sponsor and the Sponsor designee by facsimile within 24 hours of the Investigators' knowledge of the event.

7.3 Adverse Events Monitoring

Safety review will be performed by the Sponsor designee once SAE forms have been received and the CRFs electronically completed by the Investigator.

At every monitoring visit performed by the designed clinical research monitor in charge of the study, the consistency between the CRF/SAE data reported to the Sponsor / Sponsor designee and the patient's source data will be reviewed. When a discrepancy is found during the review, data will be amended/updated in the CRF and the SAE form/information reported to the Sponsor and the Sponsor designee (when applicable), according to source data.

SAEs will be continuously collected, assessed and reported throughout all the study as per the applicable legislation by the Sponsor designee. Periodic safety reviews of SAE reports including events of special interest (e.g., neutropenia and thrombocytopenia) are to be conducted and documented by the Sponsor designee.

Non-serious AEs will be verified during monitoring visits by the clinical trial monitor, who will discuss them with the Investigators, if applicable. The personnel in charge of this process are defined in the section "*Study Contacts*" of this protocol. Periodic safety review of safety data from the clinical database, i.e. AEs and laboratory data, will be performed along the study by the Sponsor Pharmacovigilance, Clinical Oncology and Data Management departments.

8. PHARMACEUTICAL INFORMATION

8.1 Eribulin

8.1.1 **Description**

- Generic name (INN): eribulin mesylate
- Chemical name (USAN/INN): (2*R*,3*R*,3a*S*,7*R*,8A*S*,9*S*,10a*R*,11*S*,12*R*,13a*R*,13b*S*,15*S*,18*S*,21*S*,24*S*,26*R*,28*R*,29a*S*)-2-[(2*S*)-3-Amino-2-hydroxypropyl]3-methoxy-26-methyl-20,27-dimethyli denehexacosahydro-11,15:18,21:24,28-triepoxy-7,9-ethano-12,15-methano-9*H*,15,*H*furo[3,2-*i*]furo[2',3':5,6]pyrano[4,3-*b*][1,4]dioxacyclopentacosin 5(4*H*)-one methanesul fonate (salt).
- Molecular formula: C41H63NO14S (C40H59NO11・CH4O3S)
- Molecular weight: 826.00
- Labeling will include the manufacturer, chemical name/identifier, lot/batch number, storage conditions, expiration date
- A list of the adverse events and potential risks associated with the investigational in this study can be found in Section 7.1.
- The PK of Eribulin is linear with mean elimination half-life of 40 hours, a mean volume of distribution of 43 L/m2 to 114 L/m2 and mean clearance of 1.16 L/hr/m2 to 2.42 L/hr/m2 over the dose range of 0.25 mg/m2 to 4.0 mg/m2 The human plasma protein binding of eribulin at concentrations of 100 ng/mL to 1,000 ng/mL ranges from 49% to 65% Eribulin exposure after multiple dosing is comparable to that following a single dose. No accumulation of eribulin is observed with weekly administration.
- Unchanged eribulin was the major circulating species in plasma following administration of 14C-eribulin to patients. Metabolite concentrations represented <0.6% of parent compound, confirming that there are no major human metabolites of eribulin.
- Cytochrome P450 3A4 (CYP3A4) negligibly metabolizes eribulin in vitro. Eribulin inhibits CYP3A4 activity in human liver microsomes, but it is unlikely that eribulin will substantially increase the plasma levels of CYP3A4 substrates. Eribulin shows no induction potential for CYP1A, CYP2C9, CYP2C19, and CYP3A in primary human hepatocytes. No significant inhibition of CYP1A2, CYP2C9, CYP2C19, CYP2D6, or CYP2E1 was detected with eribulin concentrations up to 5 μM in pooled human liver microsomes. In vitro drug interaction studies indicate that eribulin does not inhibit drugs that are substrates of these enzymes and it is unlikely that eribulin will affect plasma levels of drugs that are substrates of CYP enzymes. Eribulin is a substrate and a weak inhibitor of the drug efflux transporter P-gp in vitro
- Eribulin is eliminated primarily in feces unchanged. After administration of 14C-eribulin to patients, approximately 82% of the dose was eliminated in feces and 9% in urine. Unchanged eribulin accounted for approximately 88% and 91% of the dose in feces and urine, respectively.

8.1.2 **Form**

Eribulin will be supplied to the sites in glass vials containing 1.0 mg Eribulin mesylate in 2.0 mL of clear, colorless, and sterile solution.

8.1.3 **Storage and Stability**

Study drug will be stored in accordance with the labeled storage conditions. Temperature monitoring is required at the storage location to ensure that the study drug is maintained within an established temperature range. The PI or designee is responsible for ensuring that the temperature is monitored throughout the total duration of the study and that records are maintained; the temperature will be monitored continuously by using either an in-house validated data acquisition system, a mechanical recording device, such as a calibrated chart recorder, or by manual means, such that minimum and maximum thermometric values over a specific time period can be recorded and retrieved as required.

8.1.4 **Compatibility**

Do not mix with other drugs or administer with dextrose- containing solutions

8.1.5 **Handling**

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

8.1.6 **Availability**

Eisai will package eribulin as open-label supplies. Eribulin will be supplied to the sites in glass vials containing 1.0 mg Eribulin mesylate in 2.0 mL of clear, colorless, and sterile solution. Excipients of the Eribulin formulation are ethanol, hydrochloric acid, sodium hydroxide, and water for injection.

8.1.7 **Preparation**

The amount of Eribulin required will be withdrawn from the appropriate number of vials (each vial contains 1.0 mg of Eribulin mesylate in 2.0 mL solution [concentration: 0.5 mg/mL]) into a syringe.

Store undiluted HALAVEN in the syringe for up to 4 hours at room temperature or for up to 24 hours under refrigeration (40°F or/ 4°C). Store diluted solutions of HALAVEN for up to 4 hours at room temperature or up to 24 hours under refrigeration.

8.1.8 **Administration**

Before dose administration, the amount of study treatment needed for each subject will be calculated by the Dubois formula or institutional standard of care at day 1 of each cycle.

The dose of study treatment, time of administration, volume infused, and start/stop dates of the administration of study treatment will be recorded on the eCRFs. In addition, any changes to administration such as dose reduction, delay, or interruption will be adequately documented in the subject's drug administration chart and recorded on the eCRFs.

Treatment criteria according to section 5.2 must be met prior to the infusion.

Subjects will receive Eribulin mesylate at a dose of 1.4 mg/m2 as an IV infusion over 2-5 minutes on Days 1 and 8 of every 21-day cycle.

8.1.9 **Ordering**

Eribulin mesylate will be supplied in glass vials containing 1.0 mg Eribulin in 2.0 mL of clear, colorless, and sterile solution by Eisai.

Drug supply will be requested by the Drug Supply Re-ordering Form (appendix B).

8.1.10 **Accountability**

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

8.1.11 **Destruction and Return**

All unused drug supplied by Eisai will be properly destroyed at the study site. Documentation of this procedure will be provided to Eisai by the study team on request.

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

9.1 Biomarker Studies

As angiosarcomas, and other vascular sarcomas, are ultra-orphan diseases with incidences of less than 200 patients per year, very little is known about the genomic drivers behind these malignancies. Moreover, mechanisms of the vascular effects of Eribulin remain elusive. Thus, to this end we believe it will be critical to capture genomic data to help us understand this disease and any indicators of resistance or sensitivity to chemotherapies, such as Eribulin. In this protocol we will collect circulating cell free DNA (cfDNA) for whole exome sequencing (WES) as well as pre and on treatment biopsies for WES and RNA seq.

We hypothesize that these exploratory analyses will provide insight into angiosarcoma and EHE oncogenesis, as well Eribulin anti-cancer activity.

9.2 Laboratory Correlative Studies

9.2.1 *Peripheral blood cfDNA*

9.2.1.1 Collection of Specimen(s): Two tubes of peripheral blood will be collected in Streck tubes (10 mL per tube) on each cycle day 1 prior to Eribulin infusion.

9.2.1.2 Shipping of Specimen(s): The samples will be immediately sent to the Broad Institute by courier at room temperature.

9.2.1.3 Site(s) Performing Correlative Study: Analysis of cfDNA by WES will be performed at the Broad Institute. Within three hours of collection, we will perform high-speed centrifugation to separate plasma and cells. We will extract cfDNA using magnetic bead-based protocols. To qualify the cfDNA obtained from each blood biopsy for WES, we will first assess the purity of tumor-derived cfDNA using ultra-low-pass whole genome sequencing (ULP-WGS).

The ULP-WGS is a low-cost approach to nominate samples containing sufficient fraction of tumor-derived DNA for WES. Briefly, cfDNA samples will be subjected to whole genome sequencing to an average of 0.1X genome-wide sequencing coverage. Samples with fewer than 150,000 reads (0.005X) will be excluded due to insufficient coverage for analysis. We will utilize the statistical approach from the HMMcopy software(28) to correct for GC-content and mappability (sequence uniqueness) biases in read counts within genomic bins of 1Mb, which substantially improves signal to noise ratio. Next, we will use a modified approach from the TITAN(29) framework to perform segmentation, copy number prediction, and purity and ploidy estimation. This approach is optimized for increased sensitivity to detect events from low purity tumor-derived DNA in the absence of a control sample. Benchmarking of the purity estimates has been carried out using simulation of tumor-normal mixing and comparison to corresponding WES data, revealing a robust lower estimation level of 5% purity. As described in our Preliminary Data, we expect at least 50% of blood biopsies to have sufficient tumor-derived cfDNA to proceed with WES.

In those samples deemed by ULP-WGS to be adequate for WES, the exome will be selected using solution-phase hybrid capture and sequencing will be performed using the Illumina HiSeq2500, as described above. WES data will be analyzed for base substitutions, small insertions/deletions, and copy number alterations(30-34).

9.2.2 *Sarcoma whole exome (WES) and transcriptome sequencing (RNA Seq)*

9.2.2.1 Core or excisional biopsies will be performed 1. After registration but before C1D1 and 2. Prior to the first imaging and C3D1

9.2.2.2 Handling of Specimens(s): Specimens will be divided either at the bedside or in the frozen laboratory by pathology for correlative studies and submission to the MGH or BWH pathology. Research samples will be placed in 2 mL cryovials and packaged for shipping on dry ice.

9.2.2.3 Shipping of Specimen(s): Research specimens will be couriered to the Broad Institute immediately after processing.

9.2.2.4 Site(s) Performing Correlative Study: Analysis of DNA by WES will be performed at the Broad Institute

9.2.2.5 Methods: Our approach will characterize specimen "sets" consisting of pre-treatment and, when applicable, post-treatment tumor samples from each patient. Genomic DNA and polyadenylated RNA will be extracted from tumor using standard techniques, and WES and RNA-seq will be performed by the Broad Institute Genomics Platform. The exome will be selected using solution-phase hybrid capture(35-38) and sequencing will be performed using the Illumina HiSeq2500, yielding an average depth of coverage of >150-fold. WES data will be analyzed for base substitutions, small insertions/deletions, and copy number alterations(30-34). For RNA-seq, strand-specific cDNA libraries will be generated, and Illumina sequencing libraries will be prepared following established protocols. Each sample will be sequenced with an Illumina HiSeq2500. RNA-seq data will be analyzed for fusions, chimeric read-through transcripts, point mutations, and instances of alternative splicing. RNA-seq data will also be used to determine gene expression levels. Data from RNA-seq will be combined with the WES results to nominate candidate genomic alterations. Differences in genomic alterations between pre- and post-treatment tissues will be analyzed by algorithms described below.

The purpose of RNA-seq here is three-fold. First, RNA-seq can identify structural genomic changes that cannot be detected by WES, such as rearrangements and alternatively spliced isoforms(39). Second, expression data from RNA-seq can be cross-referenced with WES data to determine if genomic alterations detected by WES are expressed. Finally, the transcriptional profiles can be used to classify tumors based on signatures as described below. In particular, comparison of signatures between pre-treatment samples and post-treatment samples should identify genes/signatures whose expression changes can then be correlated with treatment.

9.2.3 Analysis of WES and RNA Seq Data

Analysis of the sequencing data will be performed in conjunction with the Cancer Genome Analysis group (CGA) of the Broad Institute. The CGA group has developed a wide range of computational tools designed to identify distinct classes of genomic alterations in individual tumors, and incorporated them into a single pipeline called FireHose. In total, FireHose accomplishes the following: (i) direct comparison of matched tumor and normal samples, (ii) detection of events that may be present at widely different ratios (rather than 0, 50 or 100% for germline alleles), (iii) detection of amplifications, deletions and rearrangements, and (iv)

overcoming contamination from admixture of normal tissue. Specifically, SNVs and indels are identified using MuTect(30) and indelocator(31), respectively. Copy number alterations (CNAs) are identified using ReCapSeg (unpublished). RNA-seq data will be analyzed using dRanger which identifies pairs of reads with abnormal distance and orientation compared to the distribution in the library, and uses a 'split-read aligner' to characterize the breakpoints to the base-level level. A variation on this approach can be used to identify alternatively spliced products or gene fusions present in RNA-seq data.

To annotate the results of the various calling methods for biological significance, one first models the background mutational processes (and their rates) and then calculates a p-value, i.e. the probability to generate from the background model a set of alterations that are at least as extreme as the observed ones. Finally, one needs to correct for multiple hypotheses testing (all genes, all SNPs or all pathways, depending on the events that are analyzed), which we perform by calculating False Discovery Rate q-values using the Benjamini-Hochberg procedure(32). Two methods have been developed that follow this approach. Recurrent CNAs are identified using the GISTIC2.0 algorithm(33), a tool that detects significant copy-number alterations, distinguishing between chromosome arm-level events and focal events. Recurrent SNV and indels in are identified using MutSigCV(34), an algorithm that analyzes mutation data in genes and pathways, incorporates the background mutation rates of different categories of mutations (e.g. mutations in CpGs, other Cs or Gs, A/T, indels, etc.) and identifies genes whose mutations are unlikely to have emerged by chance.

10. STUDY CALENDAR

Scans and x-rays must be done ≤4 weeks prior to the start of therapy. Screening laboratory tests and ECG will be performed within 15 days prior to the start of therapy. In the event that the participant's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy. Laboratory testing must be repeated if done more than 7 days prior to day 1.

Assessments must be performed prior to administration of any study agent. Study assessments and agents should be administered within ± 3 days of the protocol-specified date, unless otherwise noted. Dosing delays beyond noted windows for non-clinical reasons (e.g. weather, holidays) are permitted with approval of the overall PI.

	Pre-study	Cycle 1			Cycle 2 and beyond			Off Study[H]
		Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	
Eribulin[A]		X	X		X	X		
Informed consent	X							
Pathology Review[B]	X							
Demographics	X							
Medical Hx	X							
Concurrent Meds	X	X	X		X	X		
Physical Exam	X	X	X		X	X		X
Vital Signs including weight	X	X	X		X	X		X
Height	X							
ECOG PS	X	X			X			X
Laboratory testing[C]	X	X	X		X	X		X
ECG	X[I]				X[I]			
Adverse Event Evaluation		X	X	X	X	X	X	X
Tumor Measurements[D]	X	Every 6 weeks for the first 7 cycles (before C3, C5, C7) and then every 9 weeks onward (before C10, C13, etc)						
Urine or serum B-HCG[E]	X							
Required Biopsy[F]	X						X	
Blood sample for cfDNA[G]		X			X			

A- Eribulin will be administered of 1.4 mg/m2 as an IV infusion over 2-5 minutes on Days 1 and 8 of every 21-day cycle

B- Pathology review any time prior to the study

C- CBC/diff, chemistries (sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, calcium, phosphorus, magnesium, total bilirubin, alkaline phosphatase, AST, ALT, total protein, albumin); urinalysis only required on prescreening testing and PT/PTT only required on prescreening testing and before Biopsy 2. Screening laboratory tests will be performed within 15 days prior to Day 1 of treatment

D- The modality (e.g. CT, MRI) should be consistent through the protocol. The window is +/- 1 week for the first 3 sets of scans/imaging then +/- 2 weeks thereafter; baseline tumor measurements should be performed within 28 days prior to Day 1 of treatment

E- Urine or serum beta-HCG Must be repeated if not performed within 72 hours of cycle 1 day 1
F- Biopsy 1 will occur after registration and prior to C1D1. Biopsy 2 will occur prior to the first imaging and C3D1. Platelet count and PT/PTT required pre biopsy 2 or per institutional standards. When possible the biopsy should be multiple cores or excisional. If the biopsy is considered unsafe the requirement may be waived after discussion with the overall PI.
G- cfDNA will be collected after registration (prior to C1D1) and every D1 thereafter
H- See section 5.8 for AE and SAE follow-up.
I- ECG monitoring only required for screening, C2D1 and C3D1 unless the criteria for continued monitoring are met as per section 6.3. ECG does not need to be repeated on C1D1 if obtained within 15 days of C1D1, unless clinically indicated.

11. MEASUREMENT OF EFFECT

11.1 Antitumor Effect – Solid Tumors

For the purposes of this study, participants should be re-evaluated for response every 6 weeks for the first 7 cycles (before C3, C5, C7) and then every 9 weeks onward (before C10, C13, etc.).

Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [*Eur J Ca* 45:228-247, 2009]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

11.1.1 Definitions

Evaluable for Target Disease response. Only those participants who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for target disease response. These participants will have their response classified according to the definitions stated below. (Note: Participants who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response. Participants who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

11.1.2 Disease Parameters

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 by chest x-ray or ≥10 mm with CT scan, MRI, or calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

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.*

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, abdominal masses (not followed by CT or MRI), and cystic lesions are all considered non-measurable.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same participant, these are preferred for selection as target lesions.

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 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), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow up.

11.1.3 Methods for Evaluation of Disease

All measurements should be taken and recorded in metric notation using a ruler, calipers, or a digital measurement tool. 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.

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 unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions. Clinical lesions will only be considered measurable when they are superficial (*e.g.*, skin nodules and palpable lymph nodes) and ≥10 mm in diameter as assessed using calipers (*e.g.*, skin nodules). 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. This guideline has defined measurability of lesions on CT scan based on the assumption that CT thickness is 5mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size of a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (*e.g.* for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

FDG-PET. While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

(a) Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.

(b) No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.

(c) FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be

prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

Note: A 'positive' FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

PET-CT. At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.
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Ultrasound. Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later data and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure from CT, MRI may be used instead of CT in selected instances.

Endoscopy, Laparoscopy. The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

Cytology, 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.

11.1.3.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

11.1.3.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis).

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

Non-CR/Non-PD: 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. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

11.1.3.3 Evaluation of New Lesions

The finding of a new lesion should be unequivocal (i.e. not due to difference in scanning technique, imaging modality, or findings thought to represent something other than tumor (for example, some 'new' bone lesions may be simply healing or flare of pre-existing lesions). However, a lesion identified on a follow-up scan in an anatomical location that was not scanned at baseline is considered new and will

indicate PD. If a new lesion is equivocal (because of small size etc.), follow-up evaluation will clarify if it truly represents new disease and if PD is confirmed, progression should be declared using the date of the initial scan on which the lesion was discovered.

11.1.3.4 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.

For Participants with Measurable Disease (*i.e.*, Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥4 wks Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once ≥4 wks from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.
** Only for non-randomized trials with response as primary endpoint.
*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

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

For Participants with Non-Measurable Disease (*i.e.*, Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated

Unequivocal PD	Yes or No	PD
Any	Yes	PD
* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised		

11.1.4 Duration of Response

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, or death due to any cause. Participants without events reported are censored at the last disease evaluation).

Duration of overall complete response: The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented, or death due to any cause. Participants without events reported are censored at the last disease evaluation.

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, including the baseline measurements.

11.1.5 Progression-Free Survival

Overall Survival: Overall Survival (OS) is defined as the time from randomization (or registration) to death due to any cause, or censored at date last known alive.

Progression-Free Survival: Progression-Free Survival (PFS) is defined as the time from randomization (or registration) to the earlier of progression or death due to any cause. Participants alive without disease progression are censored at date of last disease evaluation.

Time to Progression: Time to Progression (TTP) is defined as the time from randomization (or registration) to progression, or censored at date of last disease evaluation for those without progression reported.

12. DATA REPORTING / REGULATORY REQUIREMENTS

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

12.1 Data Reporting

12.1.1 Method

The Office of Data Quality (ODQ) will collect, manage, and perform quality checks on the data for this study.

12.1.2 Responsibility for Data Submission

Investigative sites within DF/HCC or DF/PCC are responsible for submitting data and/or data forms to the Office of Data Quality in accordance with DF/HCC SOPs.

12.2 Data Safety Monitoring

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

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

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

This is a pilot phase II clinical trial with a primary endpoint of objective response per RECIST 1.1 among patients with metastatic or unresectable angiosarcoma and EHE treated with Eribulin.

For the primary endpoint of best overall objective response with a null hypothesis of 10% and an alternative hypothesis of 35%, we would need 16 patients in a single-stage design. We will need to observe at least 4 responses out of 16 patients to accept the treatment. The power for this design is 87% using the exact binomial distribution. The operating characteristics of this design are calculated using one-sided 10% type I error.

13.2 Sample Size, Accrual Rate and Study Duration

For the primary endpoint of best overall objective response with parameters as described in 13.1, a total sample size of 16 patients is needed in a single-stage design. A minimum accrual rate is

expected to be 1 subjects per two months. With a minimal accrual rate of **0.5** subjects per month, we expect the study enrollment to last approximately **32** months.

13.3 Interim Monitoring Plan

Please see section 12.1 for safety monitoring by the DF/HCC DSMB. There is no other interim monitoring plan for this pilot study.

13.4 Analysis of Primary Endpoints

The best overall objective response rate is defined as, the proportion of patients with complete [CR] or partial response [PR] per RECIST 1.1 among all eligible angiosarcoma and EHE patients treated with Eribulin. Disease assessment will be performed every 6 weeks for the first 8 cycles and then every 9 weeks or as clinically indicated thereafter. The two-sided 95% exact binomial confidence interval will be computed for objective response.

13.5 Analysis of Secondary Endpoints

There is at least 74% probability of observing one or more rare (8% true probability) events, and 93% probability of observing toxicities that have a true occurrence of at least 15%. With 16 treated patients, the maximum width of a 90% two-sided exact binomial confidence interval for any estimated adverse event proportion will be no wider than ±22%.

Progression-free survival will also be calculated. Progression-free survival, will be defined as the duration of time from start of treatment to time of progression or death, whichever occurs first and will be estimated using the Kaplan-Meier method. The disease control rate (Complete Response, Partial Response, and Stable Disease) at 24 weeks will also be calculated using the exact binomial distribution and 95% confidence interval.

We will explore the relationship between integrated biomarkers detected by cfDNA exome sequencing, WES, RNAseq expression profiling and response to Eribulin. We will use Fisher's Exact Test to assess the relationship between each biomarker and response to eribulin assuming a best overall objective response of 35%. Biomarkers may be continuous or binary. For example, with this design, assuming tissue is available on 100% of the sample, the probability of concluding eribulin response is related to the exploratory biomarker is 82%; given the unknown true response is 70% and 4% in positive marker versus negative marker patients, respectively, assuming 50% of the population is over-expressed for the biomarker or the biomarker is continuous and split at the median. The operating characteristics of this design are calculated using a two-sided exact test with 10% type I error.

We will explore the relationship between changes in the integrated biomarkers from baseline to cycle 3 due to eribulin treatment using a paired t-test. With 16 patients, we will have 81% power to detect an effect size of 0.67 using a paired t-test with a 0.1 two-sided significance level.

13.6 Reporting and Exclusions

13.6.1 Evaluation of Toxicity

All participants will be evaluable for toxicity from the time of their first treatment. All patients who receive at least one dose of Eribulin will be included in the toxicity analysis. This includes participants who receive study drug and are ultimately deemed ineligible.

13.6.2 Evaluation of the Primary Efficacy Endpoint

All participants who received at least one dose of Eribulin will be included in the overall response rate analysis. All eligible participants included in the study must be assessed for response to treatment, even if the patient is ineligible or there are major protocol therapy deviations, following intent-to-treat.

14. PUBLICATION PLAN

The results should be made public within 24 months of reaching the end of the study. The end of the study is the time point at which the last data items are to be reported, or after the outcome data are sufficiently mature for analysis, as defined in the section on Sample Size, Accrual Rate and Study Duration. If a report is planned to be published in a peer-reviewed journal, then that initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors. A full report of the outcomes should be made public no later than three (3) years after the end of the study.

15. 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.

16. APPENDIX B DRUG REORDER FORM

17. REFERENCES

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