A Non-Inferiority Study of Doxorubicin with Upfront Dexrazoxane for the Treatment of Advanced or Metastatic Soft Tissue Sarcoma

Washington University School of Medicine
Division of Oncology
660 South Euclid Avenue, Campus Box 8056
St. Louis, MO 63110

Protocol #: 201510049
Version Date: 11/01/19

Principal Investigator: **Brian A. Van Tine, M.D., Ph.D.**
Phone: (314) 747-8475
E-mail: bvantine@wustl.edu

.

Sub-Investigators	**Modality**
Ashley E. Frith, M.D.	Medical Oncology
Douglas R. Adkins, M.D.	Medical Oncology
Peter Oppelt, M.D.	Medical Oncology
Caron Rigden, M.D.	Medical Oncology
Angela Hirbe, M.D.	Medical Oncology
Jose Alvarez-Cardona, M.D.	Cardiology
John Gorcsan, M.D.	Cardiology
George Heberton, M.D.	Cardiology
Ronald J. Krone, M.D.	Cardiology
Daniel Lenihan, M.D	Cardiology
Joshua Mitchell M.D.	Cardiology
Justin Vader, M.D.	Cardiology
Kathleen Zhang, M.D.	Cardiology
Mitchell G. Scott PhD	Pathology and Immunology
Fei Wan, Ph.D.	Biostatistics

Study Drug(s): Dexrazoxane (Zinecard)

ClinicalTrials.gov#: NCT02584309

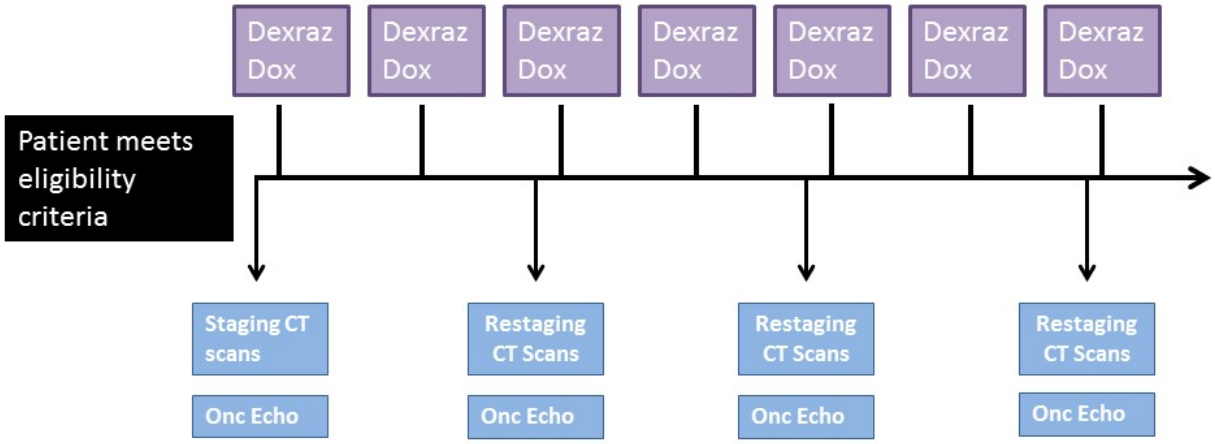
A Non-Inferiority Study of Doxorubicin with Upfront Dexrazoxane for the Treatment of Advanced or Metastatic Soft Tissue Sarcoma

Protocol Revision History

Initial Approval Version	**08/28/15**
Amendment #1 Version	**12/22/15**
Amendment #2 Version	**06/21/16**
Amendment #3 Version	**10/17/16**
Amendment #4 Version	**12/22/16**
Amendment #5 Version	**06/26/17**
Amendment #6 Version	**08/01/17**
Amendment #7 Version	**01/26/18**
Amendment #8 Version	**10/04/18**
Amendment #9 Version	**02/04/19**
Amendment #10 Version	**11/01/19**

A Non-Inferiority Study of Doxorubicin with Upfront Dexrazoxane for the Treatment of Advanced or Metastatic Soft Tissue Sarcoma

Study Schema



Glossary of Abbreviations

AE	Adverse event
ALT (SGPT)	Alanine transaminase (serum glutamate pyruvic transaminase)
AST (SGOT)	Aspartate transaminase (serum glutamic oxaloacetic transaminase)
B-HCG	Beta human chorionic gonadotropin
BNP	B-type natriuretic peptide
CBC	Complete blood count
CMP	Complete metabolic panel
CR	Complete response
CRF	Case report form
CT	Computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
DNA	deoxyribonucleic acid
DSM	Data and Safety Monitoring
ECOG	Eastern Cooperative Oncology Group
EDTA	ethylenediaminetetraacetic acid
EORTC	European Organisation for Research and Treatment of Cancer
FDA	Food and Drug Administration
HIV	Human Immunodeficiency Virus
HRPO	Human Research Protection Office (IRB)
HRV	Heart rate variability
IRB	Institutional Review Board
IULN	Institutional upper limit of normal
LIIF	Load independent index of filling
LVEF	Left ventricular ejection fraction
MRI	Magnetic resonance imaging
NCI	National Cancer Institute
OHRP	Office of Human Research Protections
OS	Overall survival
PD	Progressive disease
PFS	Progression-free survival
PI	Principal investigator
PR	Partial response
QASMC	Quality Assurance and Safety Monitoring Committee
RECIST	Response Evaluation Criteria in Solid Tumors (Committee)
RNA	Ribonucleic acid
RR	Response rate
SAE	Serious adverse event
SD	Stable disease
UPN	Unique patient number

Table of Contents

1.0 BACKGROUND AND RATIONALE

1.1 Sarcoma

Sarcomas are a heterogeneous group of rare cancers that share a common mesenchymal origin. Mesenchymal cells are derived from pluripotent embryonic connective tissue that differentiates into one or several lineages such as muscle, adipose, cartilage, nerves, vascular tissue, etc. They are broadly divided into bone sarcomas and soft tissue sarcomas. The incidence of bone and soft tissue sarcomas in 2013 was 11,410 and 3,010, respectively [1]. There are more than 50 histologic subtypes of soft tissue sarcomas, each with a distinctive clinical profile, response to treatment, and prognosis.

1.2 Treatment of Metastatic Sarcoma

Generally, localized sarcoma is treated with surgery when feasible and adjuvant radiation with or without chemotherapy. Like any other cancer, sarcomas have the ability for hematogenous spread to develop distant metastases. For the majority of patients with metastatic soft tissue sarcoma, their cancer is incurable. Therefore, the goal of treatment is to reduce cancer bulk, minimize cancer related symptoms, improve quality of life, and increase overall survival. Despite our best efforts, the median survival of patients with distant metastases is 11 to 15 months with approximately 20-25% of patients being alive at three years [2].

Most cases of advanced soft tissue sarcoma are treated with conventional chemotherapy for palliation. A common treatment regimen is single agent doxorubicin. There have been many attempts to improve upon this treatment approach with dose intensification and combination therapies. A randomized trial by EORTC Soft Tissue and Bone Sarcoma Group compared doxorubicin alone, doxorubicin plus ifosfamide, and a four-drug regimen of cyclophosphamide, vincristine, doxorubicin, and dacarbazine. The combination treatment did not significantly improve overall survival (OS), progression-free survival (PFS), or response rate (RR), and these combination regimens were significantly more toxic [3]. Another randomized phase III trial evaluated single agent doxorubicin compared to doxorubicin plus ifosfamide in advanced or metastatic soft tissue sarcoma. There was no significant difference in OS between the two treatment groups (median OS 12.8 months vs. 14.3 months, p=0.076). The combination chemotherapy group had a significantly higher PFS (7.4 months vs. 4.6 months, p 0.003) and there was a higher overall response rate in the combination treatment arm (26% vs. 14%) [4].

1.3 Anthracyclines

Doxorubicin is a commonly used chemotherapy classified as an anthracycline. It is a natural product of a fungus, *Streptomycoces peucetius* var. *caseius*, with antitumor properties. Anthracyclines have a tetracyclic ring structure attaches to a sugar and have quinone and hydroquinone moieties on adjacent rings that permit the gain and loss of electrons.

Anthracyclines have several mechanisms of antitumor activity. They bind to DNA through intercalation to block the synthesis of DNA and RNA. This leads to DNA strand scission. Another mechanism of cytotoxicity includes inhibiting topoisomerase II, resulting in impaired DNA repair. Other major cellular enzymes affected by anthracyclines include DNA and RNA polymerase. Another mechanism of cellular destruction is the generation of semiquinone free radicals and oxygen-free radical through an iron-dependent reductive process. The presence of quinone group is to allow free radical to form and accumulate in normal and malignant cells. These free radicals will not only damage malignant cells but is also thought to contribute to toxicity of normal tissue [5, 6].

1.3.1 Cardiotoxicity and Anthracyclines

The most concerning toxicity of anthracyclines is cardiotoxicity. Anthracycline-induced cardiotoxicity ranges from subclinical left ventricular dysfunction to severe cardiomyopathy with heart failure. Cardiotoxicity may occur acutely within the first week of treatment, early within one year of completing therapy, or late after one year of completing anthracycline treatment.

The cardiotoxic effects of doxorubicin have been well established in studies dating back decades [7, 8, 9]. The estimated incidence of heart failure for patients on doxorubicin receiving a cumulative dose of 400-550 mg/m^2 range widely from a relatively low 3-7% by Von Hoff et al 1979 to a significantly higher 27% by Speyer et al 1992. At higher doses around 1000 mg/m^2 as many of half of patients may experience the cardiotoxic effects [10].

The exact mechanism of anthracycline-induced cardiotoxicity is not entirely known. It has traditionally been believed that the cardiotoxic effects of have been mediated by redox cycling and the generation of reactive oxygen species; newer evidence suggests that it may be specifically mediated by topoisomerase-IIβ in cardiac myocytes [11]. An additional mechanism may include accumulation of mitochondrial iron leading to increased reactive oxygen species inside the cardiac myocyte [12].

1.4 Dexrazoxane (Zinecard)

Dexrazoxane is a derivative of ethylenediaminetetraacetic acid (EDTA) and acts as a free-radical scavenger. It chelates iron, reducing anthracycline-metal iron complexes and thus the formation of superoxide radicals [13, 14]. It has been shown to drastically reduce the incidence of anthracycline-induced cardiomyopathy in breast cancer [15, 16, 17, 18] and sarcoma [19, 20]. The use of dexrazoxane has gained widespread currency and is now considered standard of care in several treatment regimens for sarcoma and breast cancer.

The effect of dexrazoxane on the efficacy of the chemotherapy regimen remains a topic of debate. Some studies of breast cancer patients undergoing the a multi-agent chemotherapy regimen known as FAC (a combination of 5-fluorouracil, doxorubicin, and

cyclophosphamide) have shown lower response rates in patients receiving dexrazoxane [17], prompting the FDA to limit its labeling to patients who have already received a cumulative dose of doxorubicin of 300 mg/m^2. However, others have demonstrated no difference [17, 21], and while a recent Cochrane review by Val Dalen et al have shown a non-significant trend towards decreased RR (p=0.08) there was virtually no difference in PFS (p=0.89) or OS (p=0.65) [22]. Furthermore, other studies suggest that dexrazoxane may actually limit the development of resistance [23] and allow patients to receive greater total doses of doxorubicin [16, 17]. This has led some [24] to propose the initiation of dexrazoxane at doses of doxorubicin as low as 150 mg/m^2. Nonetheless this has not been adopted by any of the major guideline writing groups.

The overwhelming majority of these trials have been conducted in breast cancer patients, and there is little evidence either for or against its early use in sarcoma patients. Moreover, in most of the breast cancer trials, coadministration of doxorubicin and dexrazoxane with cyclophosphamide and 5-fluorouracil is common, creating a possible confounder not present in standard sarcoma regimens. As such, clinical trials are needed comparing the outcomes of early versus late administration of dexrazoxane in sarcoma patients.

1.5 Measurement of Cardiac Toxicity

1.5.1 Decrease in Left Ventricular Ejection Fraction

The use of echocardiography to measure left ventricular dysfunction is an evolving science. Currently the ejection fraction, measured on a 2D echocardiogram using echogenic contrast is commonly used to monitor cardiotoxicity. However, the interoperator variability is wide and the ejection fraction actually measures a late deterioration of LV function. In most cases of anthracycline cardiotoxicity, there are permanent changes in the myocardium structure before it is reflected in a reduced ejection fraction.

There are three commonly used modalities for the measurement of left ventricular (LV) function by echocardiography, the modified Simpson's biplane method, strain measures, and 3D echocardiography. Of these, the oldest and most widely used in the Simpson's biplane estimate. This method relies on the areas of elliptical discs drawn on the apical four chamber and apical two chamber views. The difference between the end-diastolic and end-systolic volumes is then used to calculate the ejection fraction. However, this method is imperfect and does not account for changes such as left ventricular hypertrophy which may see paradoxically elevated ejection fractions even in the presence of significant systolic dysfunction.

In single center studies, calculating the ejection fraction using 3D echocardiography has been recommended to be a more consistent measure of LV function. 3D echocardiography has been around for decades, but only recently have advances in technology made this modality available for routine clinical use [25, 26]. Several studies have demonstrated improved accuracy and reproducibility of ejection fractions calculated by the 3D echo compared to the traditional Simpson's

biplane method [14, 27, 28]. However, owing to its widespread use and common acceptance use as a standard measurement in most facilities, we propose a primary endpoint of LVEF by the modified Simpson's biplane method.

A more recently developed method measures left ventricular strain, strain rate, and peak myocardial velocity [29, 30, 31]. This method uses 2D echocardiography to evaluate the degree of shortening of the ventricular myocardium using the apical 2-, 3- and 4- chamber views. A recent systematic review by Thavendiranathan et al showed the efficacy of stain measurements as an early marker of LV dysfunction in patients undergoing anthracycline-based chemotherapy [32].

Left ventricular diastolic dysfunction is a relatively recently identified clinical entity affecting nearly half of all patients with cardiac dysfunction. It is characterized by abnormal relaxation of the ventricle even in the presence of a preserved ejection fraction. Traditional methods of measuring diastolic function, including mitral inflow pulsed wave Doppler and pulmonary vein Doppler, are classified as load dependent, meaning that the measurements are dependent on and will vary with the filling pressure of the heart. More recently developed methods, including tissue Doppler imaging of the mitral annulus and color M-mode, are independent of this parameter and may vary less based on patient's fluid status. These parameters will be measured and evaluated against the Ejection fraction endpoint again to determine if changes in diastolic function will be an early marker of depressed ejection fraction.

In order to quality control these measurements, carried out in our clinical echocardiographic laboratory, the echocardiographic images will be also evaluated in our core echocardiographic laboratory. The clinical decisions will be based on the clinically obtained echocardiograms in consultation with a cardio-oncologist

A separate measure of diastolic function, Load Independent Index of Filling, has been developed at Washington University [33, 34]. This can be measured using a standard echocardiogram. We propose measuring this parameter on all echocardiograms obtained during the study and evaluating whether changes in the LIIF can be an early marker of LV systolic dysfunction.

These more recent echogenic measures of LV function will be monitored to determine if they can predict LV deterioration as measured on the ejection fraction.

1.5.2 Biomarkers as Measures of Cardiotoxicity

Serum B-type natriuretic peptide (BNP), an endogenous hormone released in response to increased preload is established as a surrogate maker for heart failure [35, 36].

Cardiac troponin I, a cardiac myocyte protein and a marker for cardiac damage has long been used as marker for coronary ischemia [37, 38]. A more recently developed

high sensitivity troponin I assay has the potential to reveal myocardial damage at a lower threshold than the current clinically used troponin. It may also have the potential to detect patients at risk for developing cardiotoxicity long before it is manifested as a decrease in left ventricular ejection fraction on echocardiography.

Galectin-3 (Gal-3), a soluble B-galactosidase-binding lectin, is produced by activated macrophages and induces cardiac fibroblasts to deposit type 1 collagen. Galectin-3 production is increased prior to and after the onset of heart failure (HF) and is believed to play a role in cardiac fibrosis. Several studies have shown that elevated Galectin-3 serum concentrations are associated with an increased two year risk of HF following acute coronary syndrome, cumulative ten year incidence of HF in the Framingham Offspring Cohort, and 18 month survival among HF patients.

Heart rate variability (HRV) is well known to provide information about the autonomic status of a patient, segregating patients with excessive sympathetic tone from those with excessive vagal tone [39]. A reduced HRV has been shown to be correlated to autonomic dysfunction and increased overall mortality [40]. It has also been used for prognostic purposes in some cancers [41, 42, 43].

1.6 Amendment #3 – Addition of Olaratumab

In October 2016, olaratumab (Lartruvo) was approved by the FDA to be given in combination with doxorubicin for the treatment of soft tissue sarcomas with a histologic subtype for which an anthracycline-containing regimen is appropriate and which is not amenable to curative treatment with radiotherapy or surgery. Amendment #3 was made to the protocol to allow patients to receive this new standard of care treatment.

1.7 Amendment #9—Removal of Olaratumab

In January 2019 Eli Lilly and Company reported that the results of the Phase 3 study of olaratumab (Lartruvo), in combination with doxorubicin in patients with advanced or metastatic soft tissue sarcoma, did not confirm the clinical benefit of olaratumab in combination with doxorubicin as compared to doxorubicin alone. Therefore olaratumab is being removed from the front line standard of care regimen. Amendment #9 was made to the protocol to reflect these changes to the standard of care treatment. Section 9.1 was also updated to clarfiy 9.1 the evaluation of cardiac function using echocardiogram and the addition of 10 sarcoma banking protocol #201203042 participants to be used as controls in the secondary objectives analysis.

2.0 OBJECTIVES

2.1 Primary Objective

To determine progression-free survival (PFS) in patients with metastatic soft tissue sarcoma who are treated with doxorubicin in combination with dexrazoxane.

2.2 Secondary Objectives

1. To determine cardiac-related mortality in patients with metastatic soft tissue sarcoma who are treated with doxorubicin in combination with dexrazoxane.

2. To determine incidence of heart failure or cardiomyopathy in patients with metastatic soft tissue sarcoma who are treated with doxorubicin in combination with dexrazoxane.

3. To evaluate additional echocardiogram parameters of left ventricular ejection fraction to determine if either 3D echocardiogram or ventricular strain is able to serve as an early marker of cardiac dysfunction compared to 2d echocardiogram modified Simpson's biplane method of LVEF.

3.0 PATIENT SELECTION

At least 57 but up to 65 patients will be enrolled in this study to receive dexrazoxane concurrently with standard of care doxorubicin. At the completion of accrual, an additional 10 patients meeting the eligibility criteria below will be enrolled as a control group for the correlative studies as there is no baseline data for these tests; these patients will receive institutional standard of care therapy.

3.1 Inclusion Criteria

1. Histologically confirmed grade 2 or 3 soft tissue sarcoma that is unresectable or metastatic. Surgery for primary or metastatic disease after chemotherapy following a response is allowed. Patients with the following tumor types are eligible:
   a. Undifferentiated pleomorphic sarcoma
   b. Leiomyosarcoma
   c. Malignant fibrous histiocytoma
   d. Liposarcoma (myxoid/round cell, pleomorphic or dedifferentiated)
   e. Synovial sarcoma
   f. Myxofibrosarcoma
   g. Angiosarcoma
   h. Fibrosarcoma
   i. Malignant peripheral nerve sheath tumor
   j. Epithelioid sarcoma
   k. Unclassified high-grade sarcoma (not otherwise specified)
   l. Soft tissue sarcoma for which treatment with an anthracycline is appropriate at the approval of the PI
2. Measurable disease according to RECIST 1.1; that is, measurable disease defined as lesions that can be accurately measured in at least one dimension (longest diameter to

be recorded) as ≥ 10 mm with CT scan, as ≥ 20 mm by chest x-ray, or ≥ 10 mm with calipers by clinical exam.

3. Planning to initiate treatment with doxorubicin (starting dose of 75 mg/m^2) as routine care.
4. Prior adjuvant chemotherapy with gemcitabine and/or docetaxel/paclitaxel is allowed.
5. At least 18 years of age.
6. ECOG performance status of 0 or 1 (see Appendix A).
7. Adequate organ function defined as:
    a. Leukocytes ≥ 3,000/mcL
    b. Absolute neutrophil count ≥ 1,500/mcl
    c. Platelets ≥ 100,000/mcl
    d. Total bilirubin ≤ 1.5 x IULN
    e. AST(SGOT)/ALT(SGPT) ≤ 3.0 x IULN
    f. Creatinine ≤ IULN OR creatinine clearance ≥ 60 mL/min/1.73 m^2 for patients with creatinine levels above institutional normal. Creatinine clearance should be calculated using the actual weight from day 1 of the cycle
8. Women of childbearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control, abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while participating in this study, she must inform her treating physician immediately.
9. Able to understand and willing to sign an IRB approved written informed consent document (or that of legally authorized representative, if applicable).

3.2 Exclusion Criteria

1. Myocardial infarction within the past 12 months, or stable or unstable angina.
2. Systolic heart failure defined as left ventricular ejection fraction ≤ 45%.
3. Symptomatic valvular heart disease.
4. Prior chemotherapy for advanced or metastatic disease.
5. Known brain metastases.
6. Prior or second primary malignancies within the last two years (except carcinoma in situ of the cervix, non-metastatic prostate cancer, or basal cell or squamous cell carcinoma of the skin which were treated with local resection only; prior adjuvant androgen deprivation therapy in the case of prostate cancer is permitted, but current adjuvant androgen deprivation therapy is not).
7. Currently receiving any investigational agents.
8. A history of allergic reactions attributed to compounds of similar chemical or biologic composition to dexrazoxane or other agents used in the study.
9. Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, or psychiatric illness/social situations that would limit compliance with study requirements.
10. Pregnant and/or breastfeeding. Patient must have a negative pregnancy test within 14 days of study entry.
11. Known HIV-positivity on combination antiretroviral therapy because of the potential for pharmacokinetic interactions with dexrazoxane. In addition, these patients are at

increased risk of lethal infections when treated with marrow-suppressive therapy. Appropriate studies will be undertaken in patients receiving combination antiretroviral therapy when indicated.

12. Prior treatment with anthracyclines.

3.3 Inclusion of Women and Minorities

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

4.0 REGISTRATION PROCEDURES

Patients must not start any protocol intervention prior to registration through the Siteman Cancer Center.

The following steps must be taken before registering patients to this study:

1. Confirmation of patient eligibility
2. Registration of patient in the Siteman Cancer Center OnCore database
3. Assignment of unique patient number (UPN)

4.1 Confirmation of Patient Eligibility

Confirm patient eligibility by collecting the information listed below:

1. Registering MD's name
2. Patient's race, sex, and DOB
3. Three letters (or two letters and a dash) for the patient's initials
4. Copy of signed consent form
5. Completed eligibility checklist, signed and dated by a member of the study team
6. Copy of appropriate source documentation confirming patient eligibility

4.2 Patient Registration in the Siteman Cancer Center OnCore Database

All patients must be registered through the Siteman Cancer Center OnCore database.

4.3 Assignment of UPN

Each patient will be identified with a unique patient number (UPN) for this study. All data will be recorded with this identification number on the appropriate CRFs.

5.0 TREATMENT PLAN

5.1 Agent Administration

Dexrazoxane will be given intravenously on an outpatient basis over 15 minutes on each day that doxorubicin is given. Dexrazoxane should be given no more than 30 minutes prior to administration of doxorubicin, which is typically given on Day 1 of a 21-day cycle. Dosing is a 10:1 ratio of dexrazoxane to doxorubicin; doxorubicin will be started at 75 mg/m^2, so dexrazoxane dosing would be 750 mg/m^2. Doxorubicin is being given as routine care; the administration is not dictated per protocol. Patients who are enrolled in the control group will receive institutional standard of care therapy.

In the event of a national shortage of dexrazoxane, 72-hour infusional doxorubicin can be used instead of dexrazoxane and bolus doxorubicin.

5.2 General Concomitant Medication and Supportive Care Guidelines

Premedications and supportive care may be given routinely as needed to prevent or treat doxorubicin-related toxicities or adverse reactions.

5.3 Women of Childbearing Potential

Women of childbearing potential (defined as women with regular menses, women with amenorrhea, women with irregular cycles, women using a contraceptive method that precludes withdrawal bleeding, and women who have had a tubal ligation) are required to have a negative pregnancy test within 14 days prior to the first dose of study treatment.

Female and male patients (along with their female partners) are required to use two forms of acceptable contraception, including one barrier method, during participation in the study and for one month following the last dose of study treatment.

If a patient is suspected to be pregnant, study treatment should be immediately discontinued. In addition a positive urine test must be confirmed by a serum pregnancy test. If it is confirmed that the patient is not pregnant, the patient may resume dosing.

If a female patient or female partner of a male patient becomes pregnant during therapy or within one month after the last dose of study treatment, the investigator must be notified in order to facilitate outcome follow-up.

5.4 Duration of Therapy

If at any time the constraints of this protocol are considered to be detrimental to the patient's health and/or the patient no longer wishes to continue protocol therapy, the protocol therapy should be discontinued and the reason(s) for discontinuation documented in the case report forms.

In the absence of treatment delays due to adverse events, treatment with dexrazoxane may continue as long as treatment with doxorubicin continues or until one of the following criteria applies:

- Documented and confirmed disease progression
- Death
- Adverse event(s) that, in the judgment of the investigator, may cause severe or permanent harm or which rule out continuation of study drug
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- Suspected pregnancy
- Serious non-compliance with the study protocol
- Lost to follow-up
- Patient withdraws consent
- Investigator removes the patient from study
- The Siteman Cancer Center decides to close the study

Patients who prematurely discontinue treatment for any reason will be followed as indicated in the study calendar.

5.5 Duration of Follow-up

Patients will be followed every 3 months for up to five years or until death, whichever occurs first. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event. Patients who go off study due to toxicities will be followed until progression of their disease.

6.0 DOSE DELAYS/DOSE MODIFICATIONS

Dosing of dexrazoxane should be consistent with dosing of doxorubicin to maintain the 10:1 ratio; if dosing of doxorubicin is reduced as part of the patient's routine care, dosing of dexrazoxane should be reduced proportionally.

Dosing of dexrazoxane should be reduced in patients with moderate to severe renal impairment (creatinine clearance levels < 40 mL/min) by 50%, i.e., reducing the dexrazoxane to doxorubicin ratio from 10:1 to 5:1.

In the event of a national shortage of dexrazoxane, 72-hour infusional doxorubicin can be used in place of dexrazoxane and bolus doxorubicin.

All dose reductions should be discussed with the Principal Investigator.

7.0 REGULATORY AND REPORTING REQUIREMENTS

The entities providing oversight of safety and compliance with the protocol require reporting as outlined below.

The Washington University Human Research Protection Office (HRPO) requires that all events meeting the definition of unanticipated problem or serious noncompliance be reported as outlined in Section 7.2.

7.1 Definitions

7.1.1 Adverse Events (AEs)

Definition: any unfavorable medical occurrence in a human subject including any abnormal sign, symptom, or disease. For the purposes of this study, adverse events related to doxorubicin are not considered reportable. However, all AEs will be tracked in OnCore. For purposes of data collection, the study drug is dexrazoxane.

Grading: the descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for all toxicity reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP website.

Attribution (relatedness), Expectedness, and Seriousness: the definitions for the terms listed that should be used are those provided by the Department of Health and Human Services' Office for Human Research Protections (OHRP). A copy of this guidance can be found on OHRP's website:
http://www.hhs.gov/ohrp/policy/advevntguid.html

7.1.2 Serious Adverse Event (SAE)

Definition: any adverse drug experience occurring at any dose that results in any of the following outcomes:

- Death
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant disability/incapacity (i.e., a substantial disruption of a person's ability to conduct normal life functions)
- A congenital anomaly/birth defect
- Any other experience which, based upon appropriate medical judgment, may jeopardize the subject and may require medical or surgical intervention to prevent one of the outcomes listed above

7.1.3 Unexpected Adverse Experience

Definition: any adverse drug experience, the specificity or severity of which is not consistent with the current investigator brochure (or risk information, if an IB is not required or available).

7.1.4 Life-Threatening Adverse Experience

Definition: any adverse drug experience that places the subject (in the view of the investigator) at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that, had it occurred in a more severe form, might have caused death.

7.1.5 Unanticipated Problems

Definition:

- unexpected (in terms of nature, severity, or frequency) given (a) the research procedures that are described in the protocol-related documents, such as the IRB-approved research protocol and informed consent document; and (b) the characteristics of the subject population being studied;
- related or possibly related to participation in the research ("possibly related" means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research); and
- suggests that the research places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.1.6 Noncompliance

Definition: failure to follow any applicable regulation or institutional policies that govern human subjects research or failure to follow the determinations of the IRB. Noncompliance may occur due to lack of knowledge or due to deliberate choice to ignore regulations, institutional policies, or determinations of the IRB.

7.1.7 Serious Noncompliance

Definition: noncompliance that materially increases risks, that results in substantial harm to subjects or others, or that materially compromises the rights or welfare of participants.

7.1.8 Protocol Exceptions

Definition: A planned deviation from the approved protocol that are under the research team's control. Exceptions apply only to a single participant or a singular situation.

Pre-approval of all protocol exceptions must be obtained prior to the event.

7.2 Reporting to the Human Research Protection Office (HRPO) at Washington University

The PI is required to promptly notify the IRB of the following events:

- Any unanticipated problems involving risks to participants or others which occur at WU, any BJH or SLCH institution, or that impacts participants or the conduct of the study.
- Noncompliance with federal regulations or the requirements or determinations of the IRB.
- Receipt of new information that may impact the willingness of participants to participate or continue participation in the research study.

These events must be reported to the IRB within **10 working days** of the occurrence of the event or notification to the PI of the event. The death of a research participant that qualifies as a reportable event should be reported within **1 working day** of the occurrence of the event or notification to the PI of the event.

7.3 Reporting to the Quality Assurance and Safety Monitoring Committee (QASMC) at Washington University

The PI is required to notify the QASMC of any unanticipated problem occurring at WU or any BJH or SLCH institution that has been reported to and acknowledged by HRPO as reportable. (Unanticipated problems reported to HRPO and withdrawn during the review process need not be reported to QASMC).

QASMC must be notified within 10 days of receipt of IRB acknowledgment via email to a QASMC auditor.

7.4 Timeframe for Reporting Required Events

Adverse events will be tracked for 30 days following the last day of study treatment. For the purposes of this protocol, adverse events considered possibly, probably, or definitely related to doxorubicin are not reportable.

8.0 PHARMACEUTICAL INFORMATION

8.1 Dexrazoxane (Zinecard)

In the event of a national shortage of dexrazoxane, 72-hour infusional doxorubicin can be used in place of dexrazoxane and bolus doxorubicin.

8.1.1 Dexrazoxane Description

Dexrazoxane is a cardioprotective agent for use in conjunction with doxorubicin. Chemically, dexrazoxane is (S)-4,4'-(1-methyl-1,2-ethanediyl)bis-2,6-piperazinedione. The molecular formula is $C_{11}H_{16}N_4O_4$, and its molecular weight is 268.28.

8.1.2 Clinical Pharmacology

The mechanism by which dexrazoxane exerts its cytoprotective activity is not fully understood. Dexrazoxane is a cyclic derivative of EDTA that penetrates cell membranes. Results of laboratory studies suggest that dexrazoxane is converted intracellularly to a ring-opened chelating agent that interferes with iron-mediated free radical generation thought to be responsible, in part, for anthracycline-induced cardiomyopathy.

8.1.3 Pharmacokinetics and Drug Metabolism

The pharmacokinetics of dexrazoxane can be adequately described by a two-compartment open model with first-order elimination. The mean peak plasma concentration of dexrazoxane was 36.5 µg/mL at 15 minutes after intravenous administration of 500 mg/m^2 of dexrazoxane.

Qualitative metabolism studies have confirmed the presence of unchanged drug, a diacid-diamide cleavage product, and two monoacid-monoamide ring products in the urine of animals and man.

8.1.4 Supplier(s)

Dexrazoxane is available commercially and will be billed as standard of care.

8.1.5 Dosage Form and Preparation

Dexrazoxane is available as 250 mg or 500 mg single dose vials as sterile, pyrogen-free lyophilizates.

Reconstitute dexrazoxane with Sterile Water for Injection, USP. Reconstitute with 25 mL for a dexrazoxane 250 mg vial and 50 mL for a dexrazoxane 500 mg vial to give a concentration of 10 mg/mL. Dilute the reconstituted solution further with Lactated Ringer's Injection, USP to a concentration of 1.3 to 3.0 mg/mL in intravenous infusion bags for intravenous infusion.

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. Solutions containing a precipitate should be discarded.

8.1.6 Storage and Stability

Reconstituted dexrazoxane for injection, when transferred to an empty infusion bag, is stable for 6 hours from the time of reconstitution when stored at controlled room temperature, 20° to 25°C (68° to 77°F) or under refrigeration, 2° to 8°C (36° to 46°F). DISCARD UNUSED SOLUTIONS. The reconstituted dexrazoxane for injection solution may be diluted with either 0.9% Sodium Chloride Injection, USP or 5% Dextrose Injection, USP to a concentration range of 1.3 to 5 mg/mL in

intravenous infusion bags. The resultant solutions are stable for 6 hours when stored at controlled room temperature, 20° to 25°C (68° to 77°F) or under refrigeration, 2° to 8°C (36° to 46°F). DISCARD UNUSED SOLUTIONS.

8.1.7 Administration

Administer the final diluted solution of dexrazoxane by intravenous infusion over 15 minutes before the administration of doxorubicin. DO NOT ADMINISTER VIA AN INTRAVENOUS PUSH. Administer doxorubicin within 30 minutes after the completion of dexrazoxane infusion.

8.1.8 Special Handling Instructions

Use caution when handling and preparing the reconstituted solution. The use of gloves is recommended. If dexrazoxane powder or solutions contact the skin or mucosae, wash exposed area immediately and thoroughly with soap and water. Follow special handling and disposal procedures.

9.0 CORRELATIVE STUDIES

9.1 Evaluation of Cardiac Function with Echocardiogram

Patients receiving dexrazoxane will undergo cardiac evaluation with echocardiograms every six weeks to evaluate for cardiac function.

Left ventricular ejection fraction (LVEF) will be evaluated by 2D echocardiogram by modified Simpson's biplane method will be provided to investigators within two days of the evaluation. Treatment decisions regarding cardiac safety will be determined by a decrease in LVEF by 2D echocardiogram (modified Simpson's biplane method) based on the clinical reading.

In addition, cardiac function will be evaluated using these images and interpreted by offline research echocardiographic laboratory and this interpretation will not be made available to the treating physicians, however the PI will have access for non-clinical purposes. Left ventricular systolic function will also be evaluated by 3D echocardiogram when technically possible.

Left ventricular diastolic function will be evaluated by mitral inflow pulsed wave Doppler to evaluate the mitral E velocity, mitral E deceleration time and mitral A velocity. Tissue Doppler imaging of the mitral annulus will also be evaluated. Load Independent Index of Filling (LIIF) will be measured. It should be noted that this has never been done outside a research laboratory. This measurement in this part of the protocol is experimental.

Right ventricular systolic function will be measured and cardiac valves will be evaluated. The additional cardiac measurements, specifically 3D echocardiogram calculated ejection

fraction, left ventricular strain, will be used to evaluate for an early or more precise measurement of cardiac dysfunction.

Additional controls for these analyses will be collected from the sarcoma banking study (HRPO #201203042) under a waiver of consent. Identifiers will be collected in order to perform the chart review. The chart review will be performed to assess for cardiac function and symptomatic heart failure.

9.2 Measurement of High Sensitivity Troponin-I (hscTnI) and Galectin-3

Blood will be drawn with pre-clinic blood work and 4 hours after doxorubicin exposure with each cycle of chemotherapy (collected at baseline and Day 1 of every cycle for control group up to Cycle 6). Ten mL of whole blood should be drawn into an EDTA tube. Blood samples will be sent to the lab of Mitchell Scott in pathology to be run in large batches.

9.3 Measurement of Brain-type Natriuretic Peptide (BNP)

Patients receiving dexrazoxane will have blood drawn to measure BNP. Blood will be drawn in an EDTA (lavender top) tube as a baseline measurement before Cycle 1 and then after a cumulative dose of 300mg/m^2 of doxorubicin. In patients continuing to receive doxorubicin after a cumulative dose of 300mg/m^2, a BNP will be measured every 6 weeks. A total of 2mL of blood needs to be collected in each EDTA tube or the lab may be added onto a basic metabolic panel or complete metabolic panel if done prior to chemotherapy. BNPs will be performed as a clinical test to evaluate early appearance of cardiac toxicity and clinically relevant LV systolic deterioration. An increase of BNP above normal or baseline will trigger additional evaluation of cardiac function (another echocardiogram) and possibly evaluation by cardiology per treating oncologist's discretion.

9.4 Heart Rate Variability

All patients receiving dexrazoxane will be wear a small, 3-channel Holter monitor (MyPatch,® DMS Holter, Stateside, CA) to record their continuous 24 hour electrocardiogram in order to examine their cardiac rhythm and measure heart rate variability (HRV). The patch recorder will be placed at the time of the baseline, 6 week and subsequent clinical echocardiograms by a cardiac tech in the north CDL. Patients will be instructed to remove the myPatch recorder after 24 hours. It should then be placed in an envelope provided by the study and FedEx pick-up. Once the HRV devices have been returned to the Washington University Cardiology Department, recordings will be scanned by the research technician at the HRV Lab and then overread for scanning accuracy by Dr. Phyllis Stein, Director of the HRV Lab. If any clinical abnormalities are seen on the Holter recording, Dr. Ron Krone will be notified and a copy of the Holter report provided to him.

10.0 STUDY CALENDAR

10.1 DEXRAZOXANE ARM

Baseline/screening procedures may take place up to 14 days prior to the start of treatment, except consent and scans (including the echocardiogram and Holter monitor), which may take place up to 28 days prior to the start of treatment.

	Baseline / Screening	**Day 1 of Each Cycle**	**Day 1 of Each Odd-Numbered Cycle[6]**	**End of Each Even-Numbered Cycle**	**EOT**	**F/U[3]**
Informed consent	X					
H&P, ECOG PS	X				X	X
CBC	X					
CMP	X					
β-hCG[1]	X					
2D and 3D echocardiogram	X		X[7]			
CT C/A/P	X			X	X	
Dexrazoxane[4]		X				
Blood for hscTnI and galectin-3		X[5]				
Blood for BNP[2]	X	After cumulative dose of 300 mg/m^2 of doxorubicin, and then every 6 weeks thereafter				
24-hour Holter monitor	X		X			
AE assessment	X ---------- X					

1. Women of childbearing potential only
2. Can be drawn with a BMP or CMP if being drawn for routine pre-doxorubicin labs
3. Every 3 months for up to 5 years; Patients who are removed for reasons other than progression will be followed until standard of care imaging shows progression per RECIST 1.1.
4. No more than 30 minutes before SOC doxorubicin
5. With SOC pre-treatment bloodwork and 4 hours post-doxorubicin
6. Except Cycle 1
7. -3 day window to allow for the echo to be read prior to the start of the next cycle

10.2 CONTROL ARM

Baseline/screening procedures may take place up to 14 days prior to the start of treatment, except consent and scans (including the echocardiogram and Holter monitor), which may take place up to 28 days prior to the start of treatment.

	Baseline / Screening	**Day 1 of Each Cycle**	**Day 1 of Each Odd-Numbered Cycle[6]**	**End of Each Even-Numbered Cycle**	**EOT**	**F/U[3]**
Informed consent	X					
H&P, ECOG PS	X				X	X
CBC	X					
CMP	X					
β-hCG[1]	X					
2D and 3D echocardiogram	X		X[7]			
CT C/A/P	X			X	X	
Dexrazoxane[4]	Given as SOC at cycle 5 or a cumulative dose of 350 mg/m^2					
Blood for hscTnI and galectin-3	X	X[5]				
Blood for BNP[2]	X	After cumulative dose of 300 mg/m^2 of doxorubicin, and then every 6 weeks thereafter				
24-hour Holter monitor	X		X			
AE assessment	X ---------- X					

1. Women of childbearing potential only
2. Can be drawn with a BMP or CMP if being drawn for routine pre-doxorubicin labs
3. Every 3 months for up to 5 years. Patients who are removed for reasons other than progression will be followed until standard of care imaging shows progression per RECIST 1.1.
4. No more than 30 minutes before SOC doxorubicin
5. With SOC pre-treatment bloodwork and 4 hours post-doxorubicin up until cycle 6
6. Except Cycle 1
7. -3 day window to allow for the echo to be read prior to the start of the next cycle

11.0 DATA SUBMISSION SCHEDULE

Case report forms with appropriate source documentation will be completed according to the schedule listed in this section.

Case Report Form	Submission Schedule
Original Consent Form	Prior to registration
On-Study Form	Prior to starting treatment
Treatment Form	Every cycle
Toxicity Form	Continuous
Treatment Summary Form	Completion of treatment
Follow Up Form	Every 3 months for up to 5 years
Tumor Measurement Form	Baseline, end of every even numbered cycles, EOT, and for all standard of care disease assessments done during follow up until documented progression.
Echocardiogram & Holter Monitor Form	Baseline, and Day 1 of every odd-numbered cycle starting with Cycle 3
hscTNI & Galectin-3 Form	Day 1 of every cycle
BNP Form	Baseline and Day 1 of every odd-numbered cycle starting with Cycle 5

12.0 MEASUREMENT OF EFFECT

12.1 Antitumor Effect – Solid Tumors

For the purposes of this study, patients should be re-evaluated for response every 6 weeks. In addition to a baseline scan, confirmatory scans should also be obtained not less than 4 weeks following initial documentation of objective response.

Response and progression will be evaluated in this study using the new international criteria proposed by the revised 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.

12.2 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, as >10 mm with CT scan, or >10 mm with calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

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, and abdominal masses (not followed by CT or MRI), are considered as 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 patient, 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.

12.3 Methods for Evaluation of Measurable Disease

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

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 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 slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for 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.

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.

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 date 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 at 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.

Tumor markers: Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published [JNCI 96:487-488, 2004; J Clin Oncol 17, 3461-3467, 1999; J Clin Oncol 26:1148-1159, 2008]. In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer [JNCI 92:1534-1535, 2000].

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.

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:

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

12.4 Response Criteria

12.4.1 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.

12.4.2 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).

12.4.3 Evaluation of Best Overall Response

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

For Patients 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: Patients 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 Patients 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

12.4.4 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).

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.

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.

12.4.5 Progression-Free Survival

PFS is defined as the duration of time from start of treatment to time of progression or death, whichever occurs first.

12.4.6 Response Review

It is strongly recommended that all responses be reviewed by an expert(s) independent of the study at the study's completion.

13.0 DATA AND SAFETY MONITORING

In compliance with the Washington University Institutional Data and Safety Monitoring Plan, the Principal Investigator will provide a Data and Safety Monitoring (DSM) report to the Washington University Quality Assurance and Safety Monitoring Committee (QASMC) semi-annually beginning six months after accrual has opened (if at least five patients have been enrolled) or one year after accrual has opened (if fewer than five patients have been enrolled at the six-month mark).

The Principal Investigator will review all patient data at least every six months, and provide a semi-annual report to the QASMC. This report will include:

- HRPO protocol number, protocol title, Principal Investigator name, data coordinator name, regulatory coordinator name, and statistician
- Date of initial HRPO approval, date of most recent consent HRPO approval/revision, date of HRPO expiration, date of most recent QA audit, study status, and phase of study
- History of study including summary of substantive amendments; summary of accrual suspensions including start/stop dates and reason; and summary of protocol exceptions, error, or breach of confidentiality including start/stop dates and reason
- Study-wide target accrual and study-wide actual accrual

- Protocol activation date
- Average rate of accrual observed in year 1, year 2, and subsequent years
- Expected accrual end date and accrual by cohort
- Objectives of protocol with supporting data and list the number of participants who have met each objective
- Measures of efficacy
- Early stopping rules with supporting data and list the number of participants who have met the early stopping rules
- Summary of toxicities separated by cohorts
- Abstract submissions/publications
- Summary of any recent literature that may affect the safety or ethics of the study

The study principal investigator and Research Patient Coordinator will monitor for serious toxicities on an ongoing basis. Once the principal investigator or Research Patient Coordinator becomes aware of an adverse event, the AE will be reported to the HRPO and QASMC according to institutional guidelines. All adverse events are tracked in the OnCore database.

14.0 STATISTICAL CONSIDERATIONS

14.1 Study Design

This is a two cohort, open label, nonrandomized study with a noninferiority primary endpoint. The last ten patients enrolled after accrual to the dexrazoxane treatment group has been completed will be considered a control group; the purpose of this group is to obtain control samples for some of the correlative studies. The primary endpoint will be tested only in the active treatment cohort. Toxicity and cardiac function biomarkers will be documented in both cohorts.

14.2 Primary Endpoint

We are comparing the progression-free survival (PFS) rate of patients being treated with doxorubicin in combination with dexrazoxane to a historic control established by Judson et al in 2014. PFS is defined as the time from randomization to the first occurrence of progression or death due to any cause. If no event exists, the PFS will be censored at the last scheduled disease assessment on study. Patients who have reached their maximum follow-up and have remained progression-free will be censored for progression at the date of their last disease assessment. Intention to treat principles will be used for analyzing the primary endpoint. The primary endpoint will be tested using estimates of median PFS and 95% confidence intervals derived from Kaplan-Meier models.

14.3 Secondary Endpoints

Cardiac-specific mortality will be analyzed using Kaplan-Meier models to estimate median times to these events, as well as the proportion experiencing an event at 3, 6, 9 and 12 months.

Agreement between 3D and 2d echocardiogram biomarkers will be described using Bland-Altman plots, which plot the mean of two measurements on the same individual against the difference of those measurements. If the values are reasonably Gaussian, the standard ± 2 standard deviations will be used as the limits of agreement. If the values are non-Gaussian, bootstrap estimates will be used to define the limits of agreement.

14.3.1 Sample Size and Statistical Power

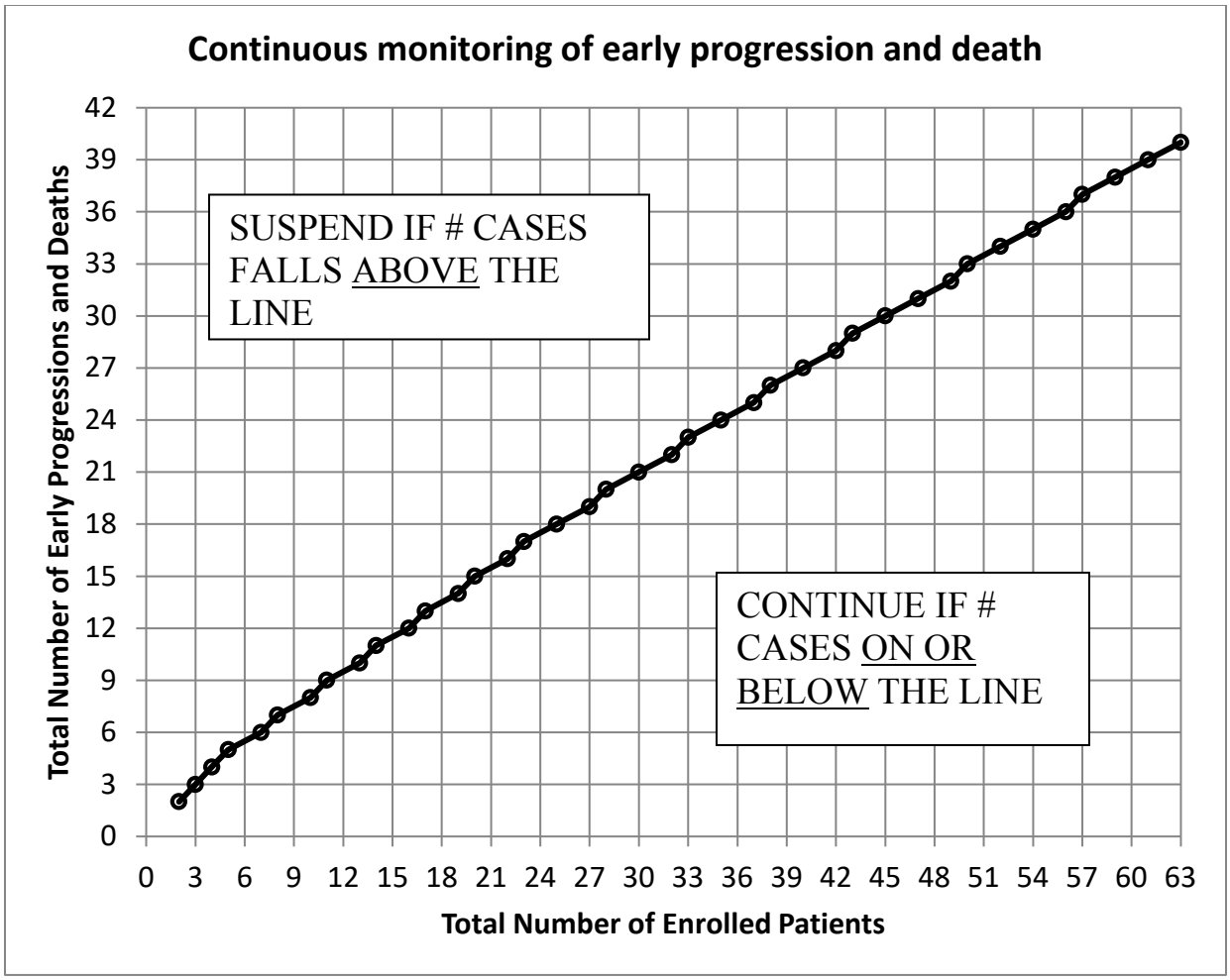
The historic control used for comparison is based on data published by Judson et al. Patients with advanced or metastatic soft tissue sarcoma treated with single agent doxorubicin had a median PFS of 4.6 months. We consider a median PFS of 3.7 months (safety margin of 20%) to be non-inferior to the PFS seen in patients treated with doxorubicin alone. We will be using a one-sided log-rank test at an alpha level of 0.05 with a power of 0.80 to determine if treatment of doxorubicin with upfront dexrazoxane is non-inferior to doxorubicin alone.

A maximum of 65 patients may be enrolled to allow for a 10% dropout rate, resulting in at least 57 patients for testing of the primary endpoint. Using 57 patients, we have 0.80 power at a 1-sided significance level of 0.05 to detect a median PFS 3.7 months compared to this historic control's PFS of 4.6 months.

14.3.2 Continuous Monitoring Rule to Evaluate for Early Progression

The expected rate is 50%, maximum allowable rate is 70%. Total number of patients is 63, including 10% for withdrawal or loss to follow up. The desired probability of stopping at a rate of 50% (incorrect early stopping) is ≤ 0.05. The desired probability of stopping at a rate of 70% (correct early stopping) is 0.80.

If the true rate is 50%, then the calculated probability of incorrect early stopping is 0.05. If the true rate is as high as 70%, the calculated probability of correct early stopping is 0.88.



A table and reference are on the following page.

Suspend for review if # early progression events or deaths EXCEEDS:	In the first ___ patients:
2	2
3	3
4	4
5	5
6	7
7	8
8	10
9	11
10	13
11	14
12	16
13	17
14	19
15	20
16	22
17	23
18	25
19	27
20	28
21	30
22	32
23	33
24	35
25	37
26	38
27	40
28	42
29	43
30	45
31	47
32	49
33	50
34	52
35	54
36	56
37	57
38	59
39	61
40	63

Reference:
Ivanova A, Qaqish BF and Schell MJ. (2005). Continuous Toxicity Monitoring in Phase II Trials in Oncology. Biometrics 61, 540-545.

14.3.3 Maximum Accrual

Maximum accrual in the treatment group is 63 patients, which includes 10% over the accrual to replace those patients deemed ineligible due to improper histology having been determined on the basis of information available prior to randomization. Accrual to the control group is 10.

15.0 REFERENCES

1. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. CA: a cancer journal for clinicians 2014;64:9-29.
2. Van Glabbeke M, van Oosterom AT, Oosterhuis JW, et al. Prognostic factors for the outcome of chemotherapy in advanced soft tissue sarcoma: an analysis of 2,185 patients treated with anthracycline-containing first-line regimens--a European Organization for Research and Treatment of Cancer Soft Tissue and Bone Sarcoma Group Study. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 1999;17:150-7.
3. Santoro A, Tursz T, Mouridsen H, et al. Doxorubicin versus CYVADIC versus doxorubicin plus ifosfamide in first-line treatment of advanced soft tissue sarcomas: a randomized study of the European Organization for Research and Treatment of Cancer Soft Tissue and Bone Sarcoma Group. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 1995;13:1537-45.
4. Judson I, Verweij J, Gelderblom H, et al. Doxorubicin alone versus intensified doxorubicin plus ifosfamide for first-line treatment of advanced or metastatic soft-tissue sarcoma: a randomised controlled phase 3 trial. Lancet Oncol 2014;15:415-23.
5. Tacar O, Sriamornsak P, Dass CR. Doxorubicin: an update on anticancer molecular action, toxicity and novel drug delivery systems. The Journal of pharmacy and pharmacology 2013;65:157-70.
6. Raj S, Franco VI, Lipshultz SE. Anthracycline-induced cardiotoxicity: a review of pathophysiology, diagnosis, and treatment. Current treatment options in cardiovascular medicine 2014;16:315.
7. Lefrak EA, Pitha J, Rosenheim S, Gottlieb JA. A clinicopathologic analysis of adriamycin cardiotoxicity. Cancer 1973;32:302-14.
8. Rinehart JJ, Lewis RP, Balcerzak SP. Adriamycin cardiotoxicity in man. Ann Intern Med 1974;81:475-8.
9. Praga C, Beretta G, Vigo PL, et al. Adriamycin cardiotoxicity: a survey of 1273 patients. Cancer treatment reports 1979;63:827-34.
10. Von Hoff DD, Layard MW, Basa P, et al. Risk factors for doxorubicin-induced congestive heart failure. Ann Intern Med 1979;91:710-7.
11. Zhang S, Liu X, Bawa-Khalfe T, et al. Identification of the molecular basis of doxorubicin-induced cardiotoxicity. Nat Med 2012;18(11):1639-42.
12. Ichikawa Y, Ghanefar M, Bayeva M, et al. Cardiotoxicity of doxorubicin is mediated through mitochondrial iron accumulation. The Journal of clinical investigation 2014;124:617-30.
13. Hasinoff BB. The interaction of the cardioprotective agent ICRF-187 [+)-1,2-bis(3,5-dioxopiperazinyl-1-yL)propane); its hydrolysis product (ICRF-198); and other chelating agents with the Fe(III) and Cu(II) complexes of adriamycin. Agents and actions 1989;26:378-85.
14. Schroeder PE, Hasinoff BB. The doxorubicin-cardioprotective drug dexrazoxane undergoes metabolism in the rat to its metal ion-chelating form ADR-925. Cancer Chemother Pharmacol 2002;50:509-13.
15. Speyer JL, Green MD, Kramer E, et al. Protective effect of the bispiperazinedione ICRF-187 against doxorubicin-induced cardiac toxicity in women with advanced breast cancer. N Engl J Med 1988;319:745-52.
16. Speyer JL, Green MD, Zeleniuch-Jacquotte A, et al. ICRF-187 permits longer treatment

with doxorubicin in women with breast cancer. J Clin Oncol 1992;10:117-27.
17. Swain SM, Whaley FS, Gerber MC, et al. Cardioprotection with dexrazoxane for doxorubicin-containing therapy in advanced breast cancer. J Clin Oncol 1997;15:1318-32.
18. Swain SM, Whaley FS, Gerber MC, Ewer MS, Bianchine JR, Gams RA. Delayed administration of dexrazoxane provides cardioprotection for patients with advanced breast cancer treated with doxorubicin-containing therapy. J Clin Oncol 1997;15:1333-40.
19. Wexler LH, Andrich MP, Venzon D, et al. Randomized trial of the cardioprotective agent ICRF-187 in pediatric sarcoma patients treated with doxorubicin. J Clin Oncol 1996;14:362-72.
20. Lopez M, Vici P, Di Lauro K, et al. Randomized prospective clinical trial of high-dose epirubicin and dexrazoxane in patients with advanced breast cancer and soft tissue sarcomas. J Clin Oncol 1998;16:86-92.
21. Seymour L, Bramwell V, Moran LA. Use of dexrazoxane as a cardioprotectant in patients receiving doxorubicin or epirubicin chemotherapy for the treatment of cancer. The Provincial Systemic Treatment Disease Site Group. Cancer prevention & control : CPC = Prevention & controle en cancerologie : PCC 1999;3:145-59.
22. van Dalen EC, Caron HN, Dickinson HO, Kremer LC. Cardioprotective interventions for cancer patients receiving anthracyclines. Cochrane Database Syst Rev 2011:CD003917.
23. Sargent JM, Williamson CJ, Yardley C, Taylor CG, Hellmann K. Dexrazoxane significantly impairs the induction of doxorubicin resistance in the human leukaemia line, K562. Br J Cancer 2001;84:959-64.
24. Swain SM, Vici P. The current and future role of dexrazoxane as a cardioprotectant in anthracycline treatment: expert panel review. Journal of cancer research and clinical oncology 2004;130:1-7.
25. Hibberd MG, Chuang ML, Beaudin RA, et al. Accuracy of three-dimensional echocardiography with unrestricted selection of imaging planes for measurement of left ventricular volumes and ejection fraction. American heart journal 2000;140:469-75.
26. Lee D, Fuisz AR, Fan PH, Hsu TL, Liu CP, Chiang HT. Real-time 3-dimensional echocardiographic evaluation of left ventricular volume: correlation with magnetic resonance imaging--a validation study. Journal of the American Society of Echocardiography : official publication of the American Society of Echocardiography 2001;14:1001-9.
27. Sapin PM, Schroder KM, Gopal AS, Smith MD, DeMaria AN, King DL. Comparison of two- and three-dimensional echocardiography with cineventriculography for measurement of left ventricular volume in patients. Journal of the American College of Cardiology 1994;24:1054-63.
28. Nosir YF, Fioretti PM, Vletter WB, et al. Accurate measurement of left ventricular ejection fraction by three-dimensional echocardiography. A comparison with radionuclide angiography. Circulation 1996;94:460-6.
29. Greenberg NL, Firstenberg MS, Castro PL, et al. Doppler-derived myocardial systolic strain rate is a strong index of left ventricular contractility. Circulation 2002;105:99-105.
30. Leitman M, Lysyansky P, Sidenko S, et al. Two-dimensional strain-a novel software for real-time quantitative echocardiographic assessment of myocardial function. Journal of the American Society of Echocardiography : official publication of the American Society of Echocardiography 2004;17:1021-9.
31. Reisner SA, Lysyansky P, Agmon Y, Mutlak D, Lessick J, Friedman Z. Global longitudinal strain: a novel index of left ventricular systolic function. Journal of the American Society of Echocardiography : official publication of the American Society of Echocardiography 2004;17:630-3.

32. Thavendiranathan P, Poulin F, Lim KD, Plana JC, Woo A, Marwick TH. Use of myocardial strain imaging by echocardiography for the early detection of cardiotoxicity in patients during and after cancer chemotherapy: a systematic review. J Am Coll Cardiol 2014;63:2751-68.
33. Kovacs SJ. Diastolic Function in Heart Failure. Clinical Medicine Insights: Cardiology 2015;9:1-7.
34. Shmuylovich L, Kovacs SJ. Load-independent index of diastolic filling: model-based derivation with in vivo validation in control and diastolic dysfunction subjects. J Appl Physiol 2006;101:92-101.
35. Tsutamoto T, Wada A, Maeda K, et al. Attenuation of compensation of endogenous cardiac natriuretic peptide system in chronic heart failure: prognostic role of plasma brain natriuretic peptide concentration in patients with chronic symptomatic left ventricular dysfunction. Circulation 1997;96:509-16.
36. McDonagh TA, Robb SD, Murdoch DR, et al. Biochemical detection of left-ventricular systolic dysfunction. Lancet 1998;351:9-13.
37. Adams JE, 3rd, Bodor GS, Davila-Roman VG, et al. Cardiac troponin I. A marker with high specificity for cardiac injury. Circulation 1993;88:101-6.
38. Antman EM, Tanasijevic MJ, Thompson B, et al. Cardiac-specific troponin I levels to predict the risk of mortality in patients with acute coronary syndromes. The New England journal of medicine 1996;335:1342-9.
39. Kleiger RE, Stein PK, Bigger JT, Jr. Heart rate variability: measurement and clinical utility. Ann Noninvasive Electrocardiol 2005;10:88-101.
40. Nolan J, Batin PD, Andrews R, et al. Prospective study of heart rate variability and mortality in chronic heart failure: results of the United Kingdom heart failure evaluation and assessment of risk trial (UK-heart). Circulation 1998;98:1510-6.
41. Lacoski SG, Jones LW, Krone RJ, Stein PK, Scott JM. Autonomic Dysfunction in Early Breast Cancer: Incidence, Clinical Importance, and Underlying Mechanisms. Submitted for Publication.
42. Scott JM, Jones LW, Hornsby WE, et al. Cancer therapy-induced autonomic dysfunction in early breast cancer: implications for aerobic exercise training. Int J Cardiol 2014;171:e50-1.
43. Fadul N, Strasser F, Palmer JL, et al. The association between autonomic dysfunction and survival in male patients with advanced cancer: a preliminary report. J Pain Symptom Manage 2010;39:283-90.

APPENDIX A: ECOG Performance Status Scale

Grade	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
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).
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.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.