A Phase II Trial of Gemcitabine plus High-Dose Ascorbate in Locally Advanced Unresectable or Metastatic Soft Tissue and Bone Sarcomas in Adults

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The Principal Investigator and all physicians responsible for patient care must have a current FDA Form 1572, Supplemental Investigator Data Form (SIDF), Financial Disclosure Form (FDF), and CV on file with the NCI. *Failure to register all appropriate individuals could delay protocol approval. If you are unsure of an investigator's status, please contact the Pharmaceutical Management Branch, CTEP at (301) 496-5725 or by e-mail at PMBRegPend@ctep.nci.nih.gov. Please indicate, on the title page, if an Associate Investigator is NOT responsible for patient care and therefore does not require a current 1572, SIDF, FDF, and CV on file.*

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Study Agent: High dose ascorbic acid
Other Agent(s): Standard chemotherapy with Gemcitabine

Investigational Agent	IND#	IND Sponsor
Ascorbic acid	137968	Mohammed Milhem, MD

1. INTRODUCTION

1.1 Disease Background

Soft tissue sarcomas (STS) are a heterogeneous group of benign and malignant tumors of various supportive tissues arising from the mesoderm. There are 80 known subtypes classified by the tissue of origin. Soft tissue sarcomas account for 1% of all human malignancies. In contrast to major advances in the biological understanding of these heterogeneous subtypes, forward progress in the systemic treatment of sarcoma has been painstakingly slow. The prognosis of patients with metastatic or recurrent disease is poor and most of them will die from tumor progression. In 2016 an estimated 12,310 new soft tissue sarcoma cases will be diagnosed in United States with approximately 4,990 deaths[1]. Taking into account differences in histology subtype, anatomic location of disease and age at disease onset, the overall median survival for patients with metastatic STS is approximately 8 to 12 months[2].

1.1.1 Ascorbate as an anti-tumor agent

Ascorbate (ascorbic acid, vitamin C, AscH) is one of the early unorthodox therapies for cancer, based on two unsupported hypotheses. McCormick postulated that ascorbate protects against cancer by increasing collagen synthesis,[3,4] while Cameron hypothesized that ascorbate could have anti-cancer action by inhibiting hyaluronidase and thereby prevent cancer spread.[5] These hypotheses were subsequently promoted by Cameron and Pauling.[6,7] Cameron and Campbell initially published case reports of 50 patients; some seemed to have benefited from high dose ascorbate.[8] Cameron and Pauling then published results of 100 patients with terminal cancer that were given intravenous ascorbate.[9] The ascorbate-treated patients were compared to 1000 retrospective controls with similar disease. Patients who received ascorbate survived on average 300 days longer than controls.[7,9] A prospective study was then conducted that randomized patients to ascorbate treatment or palliative therapy. Treated patients had a median survival of 343 days *vs*. 180 days for controls.[10] Smaller studies have also reported benefits of ascorbate.[11,12]

To test "definitively" whether ascorbate was effective, Moertel conducted two randomized placebo-controlled studies randomized to oral ascorbate; neither study showed benefit.[13,14] Subsequently, ascorbate therapy was considered ineffective. However, it was not recognized until approximately 15 years later that oral and intravenous ascorbate have strikingly different pharmacokinetics.[15,16] This difference in the administration route is key. Cameron gave patients ascorbate intravenously as well as orally, while Moertel's patients received only oral ascorbate. Thus, the issue of ascorbate in cancer treatment needs to be re-examined.

The evidence for use of ascorbate in cancer treatment falls into two categories: clinical data on dose concentration relationships and laboratory data describing potential cell toxicity with high concentrations of ascorbate *in vitro*. Clinical data show that when ascorbate is given orally, fasting plasma concentrations are tightly controlled at < 100 μM.[17] As doses exceed 200 mg, absorption decreases, urine excretion increases and ascorbate bioavailability is reduced.[15,17] In contrast, when 1.25 grams of ascorbate are

administered intravenously, concentrations as high as 1 mM are achieved. Some clinicians have infused more than 10 grams of ascorbate in cancer patients and achieved plasma concentrations of 1 to 5 mM.[18] Thus, it is clear that intravenous administration of ascorbate can yield very high plasma levels, while oral treatment does not.

Pharmacologic ascorbate concentrations have been shown to selectively kill many cancer cell types. Chen *et al*. measured cell death in 10 cancer and 4 normal cell types using 1-hour exposures to pharmacological ascorbate.[19] Normal cells were unaffected by 20 mM ascorbate whereas 5 cancer cell lines had EC_{50} values of < 4 mM, a concentration achievable by intravenous administration. In addition, cell death was independent of metal chelators, but dependent on formation of H_2O_2.[20] H_2O_2 generation was dependent on ascorbate concentration, incubation time; [H_2O_2] displayed a linear increase with [AscH] and it increased as a quadratic function of ascorbate radical, ascorbate being an electron donor to O_2 to form superoxide and, eventually, H_2O_2.

When ascorbate is infused intravenously the resulting pharmacologic concentration will distribute rapidly into the extracellular water space.[21] *In vivo*, Chen and colleagues demonstrated that intravenous injection of ascorbate (0.25-0.5 mg/g body weight) increased baseline concentrations of ascorb * n the blood and extracellular fluid to > 8 mM as well as increased formation of H_2O_2.[22] More recent studies have demonstrated that intraperitoneal doses of 4 g/kg ascorbate resulted in blood concentrations of 40 mM; tumor extracellular fluid increased to peaks of 20 mM for up to 3 hours.[17] Our data demonstrate that pharmacologic doses of ascorbate are cytotoxic to sarcoma cell lines and that it enhances chemotherapy sensitivity *in vitro* (Figure 1). Additionally, intraperitoneal administration of high dose ascorbate in combination with gemcitabine and ascorbate inhibits orthotopic sarcoma growth and decreases tumor size in mice without causing an associated toxicity (Figure 2). Combined, these studies provide a foundation for pursuing pharmacologic ascorbate as a prooxidant agent in cancer therapy and specifically in sarcoma.

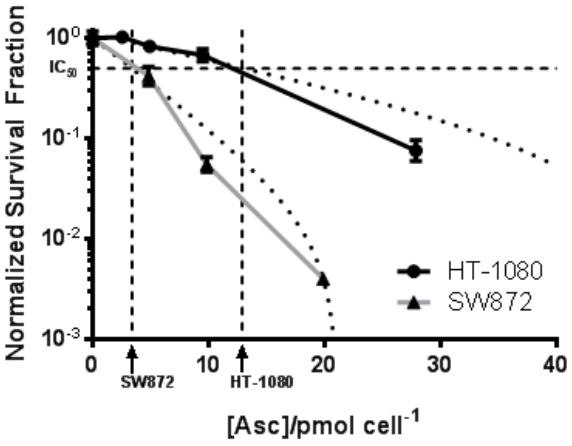


Figure 1: Ascorbate Demonstrates Dose-Dependent Cytotoxicity in Two Sarcoma Cell Lines. Clonogenic Cell survival of HT-1080 (Fibrosarcoma) and SW-872 (synovial cell sarcoma) cell lines following 1 hour exposure to increasing concentrations of ascorbate. As ascorbate concentration increased, clonogenic cell survival decreased.

Ascorbate-mediated cell death has been shown to be due to H_2O_2 generation, *via* ascorbate radical formation, with ascorbate as the electron donor.[19,20,22] When ascorbate is infused intravenously the resulting pharmacologic concentration distributes

rapidly in the extracellular water space.[22] Thus, pharmacologic ascorbate concentrations in media, as a surrogate for extracellular fluid, should generate ascorbate radical and H_2O_2. In contrast, the same pharmacologic ascorbate concentrations in whole blood generate little detectable ascorbate radical and no detectable H_2O_2.[14] This can be accounted for by efficient and redundant H_2O_2 catabolic pathways in whole blood relative to those in media or extracellular fluid. Thus, ascorbic acid administered intravenously in pharmacologic concentrations may serve as a pro-drug for H_2O_2 delivery to the extracellular milieu, but without H_2O_2 accumulation in blood.

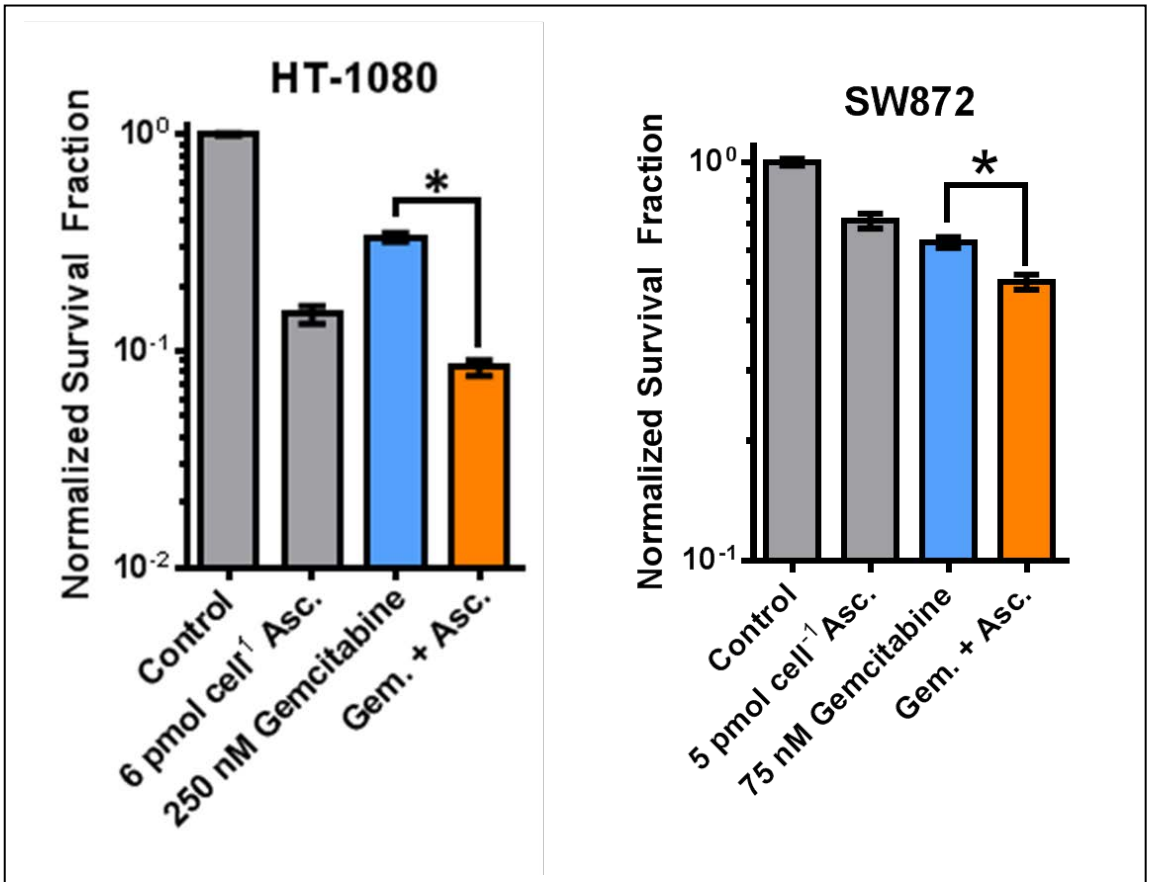


Figure 2: Pharmacological Ascorbate Sensitizes Sarcoma Cell Lines to Gemcitabine. Pharmacological levels of ascorbate enhance sarcoma cell lines, HT-1080 (Fibrosarcoma) and SW-872 (Synovial cell sarcoma) sensitivity to gemcitabine chemotherapy. Cell survival was determined using the clonogenic cell survival assay. Cell lines were treated with ascorbate and gemcitabine for 1h. Clonogenic cell survival was decreased in both cell lines when treated with gemcitabine and ascorbate. *$p < 0.05$ vs. gemcitabine alone.

1.2 **Ascorbate Preliminary Data**

1.2.1 Pre-clinical (*in vitro).*

Figure 1 demonstrates that sarcoma cell lines, HT1080 and SW-872, are sensitive to increasing doses of pharmacological ascorbate. Cells were treated with increasing doses of ascorbate for 1 hour followed by clonogenic cell survival analysis. **Figure 2** shows that pharmacological doses of ascorbate enhance the sensitivity of sarcoma cell lines, HT1080 and SW-872, to gemcitabine. Cells were treated with ascorbate and gemcitabine for one hour. Cell survival was determined by clonogenic cell survival assay. These results support the hypothesis that ascorbate enhance standard of care induced cell killing of sarcoma cells. **Figure 3** shows that ascorbate toxicity in sarcoma cell lines is dependent upon the presence of hydrogen peroxide (H_2O_2). Cells were treated with combinations of catalase and ascorbate. Catalase, which converts H_2O_2 into water and oxygen, reversed the toxicity of pharmacological doses of ascorbate. **Figure 4** shows dose response curves for three Ewing's sarcoma cell lines and a fibroblast cell line treated with different concentrations of ascorbate alone for 3 days. The dose response curves suggest that ascorbate impairs the growth of Ewing's sarcoma cells.

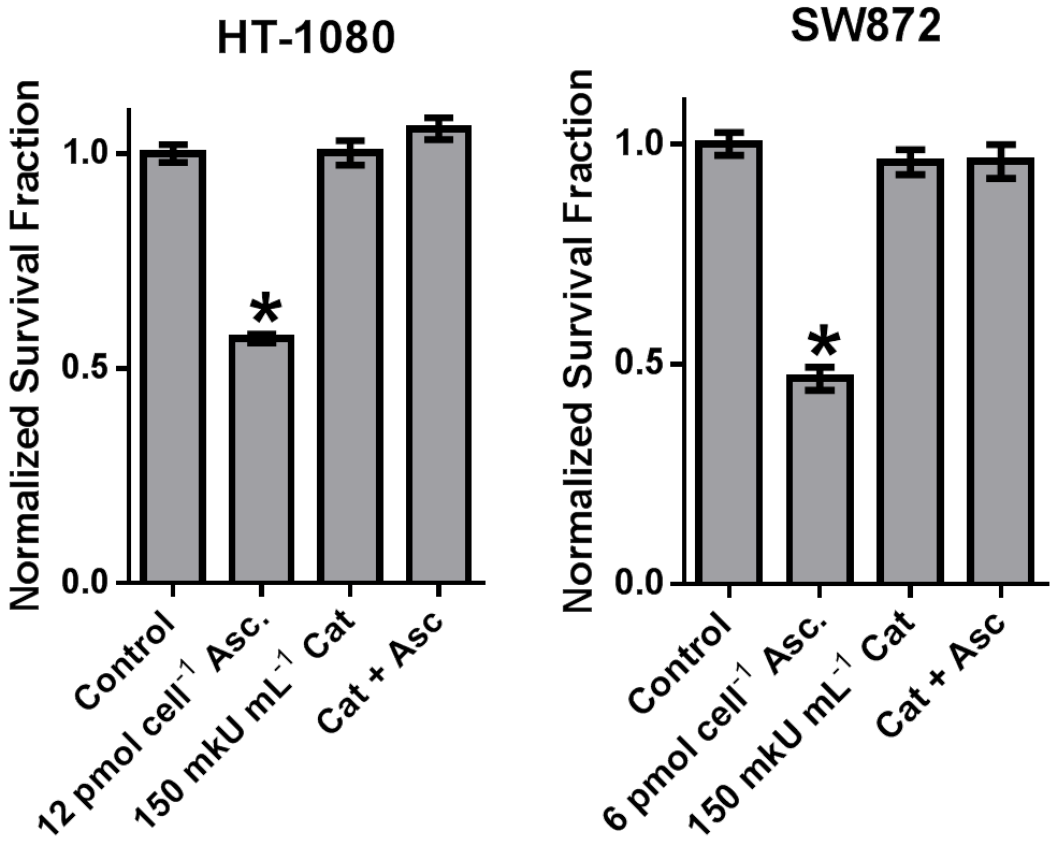


Figure 3: Pharmacological Ascorbate Cytotoxicity is Dependent upon H_2O_2. Sarcoma cell lines, HT-1080 (Fibrosarcoma) and SW-872 (Synovial cell sarcoma) were treated with combinations of pharmacological doses of ascorbate and catalase. Catalase reversed the toxic effects of ascorbate in sarcoma cell lines demonstrating that ascorbate toxicity is dependent upon H_2O_2. *$p < 0.05$ vs. control and catalse alone.

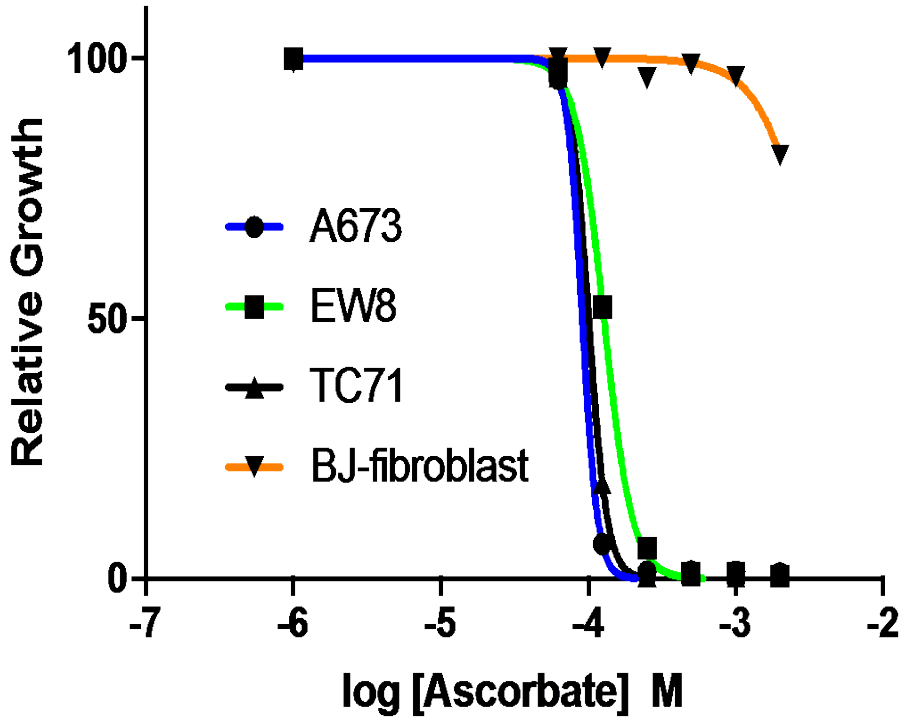


Figure 4: Ascorbate impairs the growth of Ewing sarcoma cells. Dose-response curves for three Ewing sarcoma cell lines (A673, EW8 and TC71) and a non-transformed fibroblast cell line (BJ-fibroblast) treated with different concentrations of ascorbate for three days. Cell viability was assessed using the CellTiter-Glo Luminescent Assay.

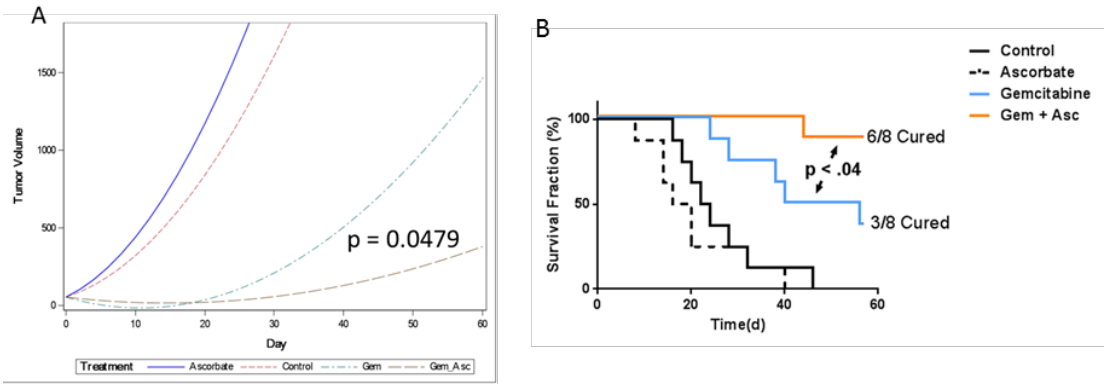


Figure 5: Pharmacological Ascorbate Enhances Gemcitabine Therapy in a Mouse Model. Sarcoma cell line cells, HT-1080 (Fibrosarcoma), were injected ino the flanks of athymic nude mice generating a xenograft. Mice were treated with combinations of saline (control), ascorbate (4g/kg IP), and gemcitabine. Mice treated with the combination of gemcitabine and ascorbate (Gem + Asc) had significantly slower tumor growth rates (A) and lived significantly longer with a higher cure rate (B) than mice treated with gemcitabine alone.

1.2.2 Pre-clinical (*in vivo*)

To determine if treatment of established sarcoma tumors in a xenograft model with ascorbate would inhibit growth, HT-1080 tumor cells (2×10^6) were delivered subcutaneously into the flank region of nude mice and allowed to grow until they reached 3 mm in greatest dimension (~ 10 days), at which time they were randomly assigned to a treatment group. This was defined as day 1 of the experiment. The animals were randomized to receive either: sham treatment (control), ascorbate (4 g/kg) i.p. given to mice every day for two weeks, gemcitabine (100mg/kg) i.p. given once weekly, and ascorbate with gemcitabine. Data from Dr. Mark Levine's laboratory have demonstrated that 4 g/kg i.p. ascorbate resulted in blood concentration from baseline of 40 μM to peaks of 40 mM while tumor extracellular fluid increased to peaks of 20 mM for up to 3 hours [personal communication].[15] The primary outcomes of interest were tumor growth over time and the potential toxicity of combining gemcitabine, radiation and pharmacological ascorbate. Tumor size (mm^3) was periodically measured via calipers throughout the experiments, resulting in repeated measurements across time for each mouse. Linear mixed effects regression models were used to estimate and compare the group-specific tumor growth curves. The observed tumor volumes and overall survival for all mice are plotted over time in **Figure 5** demonstrating that pharmacological ascorbate enhanced gemcitabine toxicity slowed tumor growth and prolonging survival in an animal model.

1.2.3 Clinical Trial Data

In a Phase I trial of intravenous ascorbic acid in patients with advanced malignancy the high-dose intravenous ascorbic acid was well tolerated when administered to patients. Adverse events and toxicity were minimal at all dose levels. Ascorbic acid concentrations reached up to 25 mmol/L in patients who received ascorbic acid of 1.5 g/kg. Of the 24 patients in the study, only 4 recorded minor adverse events including headache, dizziness

and diarrhea.[23] In another phase I trial using pharmacological ascorbate concurrently with gemcitabine in patients with locally advanced and metastatic pancreatic cancer conducted at University of Iowa Hospitals and Clinics, 9 patients received treatment. Individual doses between 50 to 125 g per infusion were given for maintenance of 350 mg/dL level in plasma. 75-g dose yielded peak plasma levels ranging between 320 and 630 mg/dL. No dose limiting toxicities or serious adverse events were reported.[24]

1.3 Rationale for the Study

1.3.1 Gemcitabine and Sarcomas

Gemcitabine (2',2'-difluorodeoxycytidine, also abbreviated dFdC) is a fluorinated analogue of the nucleoside deoxycytidine (dCTP). It is inactive in its parent form and requires successive intracellular phosphorylation of the parent drug to yield active di- and triphosphate metabolites[25]. The diphosphate form inhibits ribonucleotide reductase; the triphosphate form incorporates into DNA and competes with dCTP as a fraudulent base (dFdCTP). The inhibition of ribonucleotide reductase may allow for a self-potentiating mechanism to increase nucleotide incorporation in cells[26]. Once the gemcitabine triphosphate metabolite is incorporated into DNA, one additional nucleoside is incorporated, after which DNA chain synthesis is terminated. This "masked chain termination" leaves the fraudulent base resistant to excision repair by DNA repair enzymes, and may overcome an important mechanism of drug resistance[27].

Based on M.D. Anderson Cancer Center studies, the recommended phase II dosing for solid tumors was 790 mg/m^2 per week, weekly for 3 weeks, every 28 days. To render gemcitabine more effective, that study suggested that a longer exposure to drug may be associated with a greater antitumor effect, by maximizing the amount of gemcitabine that can accumulate intracellularly in a given time period. The proposition that prolonged-infusion gemcitabine (10 mg/m^2 per minute) results in higher clinical response rates than with bolus infusions was addressed in a randomized phase II trial in pancreatic cancer[28]. Patients were all treated on days 1, 8, and 15, every 28 days. Response rates and median survival were superior for the 43 patients who received gemcitabine in a fixed-dose-rate infusion (1,500 mg/m^2 at 10 mg/m^2 per minute), compared with the 49 patients who received gemcitabine as a 30-minute bolus infusion (2,200 mg/m^2 i.v. over 30 minutes); the median survival times were 8 months and 5 months, respectively, p = .013. Of note, despite the lower dose and lower concentration of gemcitabine triphosphate at 30 minutes, the median maximum concentration for gemcitabine triphosphate in peripheral blood mononuclear cells was greater than that for the bolus infusion (398 versus 188 μM; n = 16 patients assayed; p = .046 for the 150-minute time point)[29]. Thus, it was logical to examine a fixed-dose rate of gemcitabine in patients with sarcomas. The pharmacodynamics of gemcitabine in patients with sarcoma was consistent with those found in the pancreatic cancer study[30]. There have been a number of phase II studies in

sarcomas of soft tissue and bone that use gemcitabine as a single agent[30-33].

Table 1. Clinical studies of gemcitabine in soft tissue sarcomas

Study	Gemcitabine dose and schedule	Clinical trial design	*n* (evaluable)	Prior therapy	Responses and response rate	Response definition	Median TTP (months)	Median OS (months)
Amodio et al. [22]	1,000–1,250 mg/m^2 over 30 minutes, 3 wks on, 1 off	Phase II	18	Any	1 PR (6%, MFH)	n/a	4	n/a
Merimsky et al. [23]	1,000 mg/m^2 over 30 minutes weekly × 7, 1-wk break, then 3 wks on, 1 off[a]	Phase II	18	Yes	1 PR (6%, uterine LMS)	WHO	6.2	n/a
Spath-Schwalbe et al. [24]	200–250 mg/m^2 over 360 minutes, 3 wks on, 1 off	Phase II	18	Yes	2 PRs (11%, 2 uterine LMS)	WHO	n/a	8
Patel et al. [20]	Standard schedule; some received 1 g/m^2 over 150 minutes	Phase II	39 (non-GIST)	Any	7 PRs (18%, 4 LMS [3 uterine], 1 MFH, 1 sarcoma NOS, 1 angiosarcoma)	WHO	3.0	13.9
Okuno et al. [25]	1,250 mg/m^2 over 30 minutes, 3 wks on, 1 off	Phase II	29	First-line	1 PR (3%, uterine LMS)	WHO	6-month TTP, est. 11%	1-yr est. overall survival, 43%
Švancárová et al. [26]	1,250 mg/m^2 over 30 minutes, 2 wks on, 1 off	Phase II	32	First-line	1 PR (3%, LMS)	WHO	1.5	8.8
Okuno et al. [27]	1,250 mg/m^2 over 30 minutes, 3 wks on, 1 off	Phase II	25	None	1 PR (4%, epithelioid sarcoma)	WHO	13	15
Look et al. [28]	1,000 mg/m^2 over 30 minutes, 3 wks on, 1 off	Phase II	42 (all uterine LMS)	First-line	1 CR, 8 PRs (21%, all uterine LMS)	GOG	n/a	n/a
Hartmann et al. [29]	1,000 mg/m^2 over 30 minutes, 3 wks on, 1 off	Phase II	15	First-line	1 PR (6%, n/a)	WHO	3	6
Von Burton et al. [30]	Standard schedule	Phase II	36	None	3 PRs (8%, 1 MFH, 1 LMS, 1 sarcoma NOS)	SWOG	2	6
Wagner-Bohn et al. [31]	1,200 mg/m^2 over 30 minutes, 3 wks on, 1 off	Phase II	20 (pediatric diagnoses)	Any	0	RECIST	1.5	n/a
Maki et al. [41]	1,200 mg/m^2 over 120 minutes	Randomized phase II (one arm)	49	0–2	4 (9%, 1 LMS, 2 MFH, 1 other)	RECIST	3.0	11.5

[a]Indicated as "standard schedule" in remainder of the table.
Abbreviations: CR, complete response; est., estimated; GIST, gastrointestinal stromal tumor; GOG, Gynecological Oncology Group; LMS, leiomyosarcoma; MFH, malignant fibrous histiocytoma (undifferentiated high-grade pleomorphic sarcoma); n/a, not available; NOS, not otherwise specified; OS, overall survival; PR, partial response; RECIST, Response Evaluation Criteria for Solid Tumors; SWOG, Southwest Oncology Group; TTP, time to progression; WHO, World Health Organization.

1.3.2 Combination of ascorbate with chemotherapy

Metastatic soft tissue and bone sarcoma is associated with poor overall survival. We propose to investigate an entirely new approach, using pharmacological ascorbate, combined with chemotherapy to treat metastatic sarcoma. Intravenous ascorbate (*i.e.,* ascorbic acid, vitamin C), but not oral ascorbate, produces high plasma concentrations, which are in the range that can be cytotoxic to tumor cells. Though ascorbate has been utilized in cancer therapy, few studies have investigated intravenous delivery of ascorbate. Preliminary studies from our group have demonstrated that ascorbate induces cytotoxicity in fibrosarcoma cells. Phase I clinical trial showed the combination of high dose ascorbate with gemcitabine therapy in metastatic and node-positive pancreatic cancer improved the toxicity profile associated with this chemotherapy regimen. Proceeding to a phase II clinical trial to study efficacy of high dose ascorbic acid in combination with gemcitabine chemotherapy in metastatic soft tissue and bone sarcoma patients is a logical next step in evaluation.

2. OBJECTIVES

2.1 **Primary Objective:** Determine initial tumor response per RECIST 1.1 criteria.

2.2 **Secondary Objective**

2.2.1 Determine the median time to progression for patients with unresectable or metastatic soft tissue and bone sarcoma treated with high dose ascorbate when administered intravenously concurrently with gemcitabine

2.2.2 Determine the progression free survival and overall survival of patients with unresectable or metastatic soft tissue and bone sarcoma treated with high dose ascorbate when administered intravenously concurrently with gemcitabine

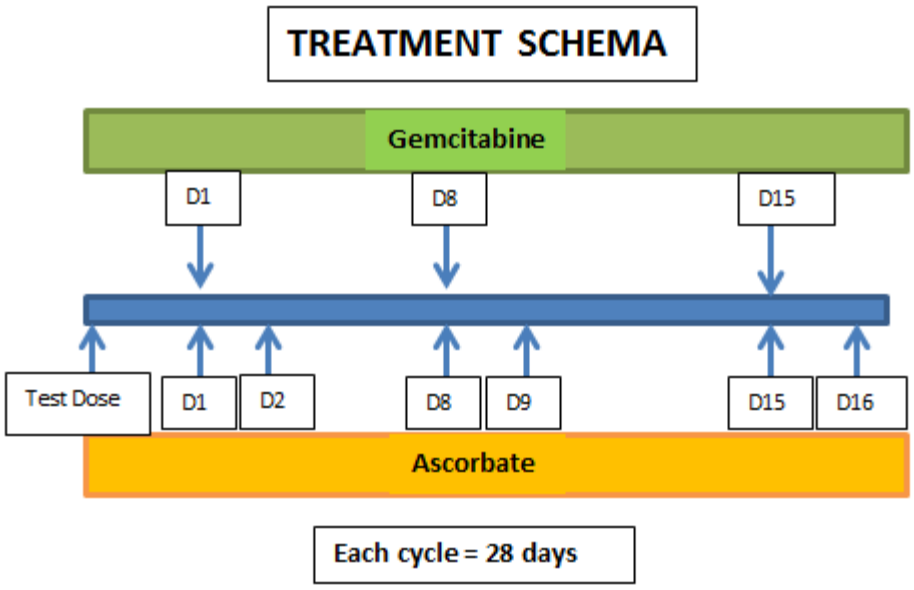
2.2.3 To evaluate the safety and tolerability of this regimen

3. OVERALL STUDY DESIGN AND SCHEMA

Phase II

The primary objective of the Phase II portion of this study is to evaluate preliminary evidence of anti-tumor activity of intravenous ascorbate in combination with gemcitabine. The primary endpoint of interest is objective response rate defined as the percentage of patients with complete response (CR) or partial response (PR) per RECIST 1.1. Gemcitabine monotherapy has been estimated to confer a 10% response rate in the treatment of metastatic osteogenic bone and 17% in soft tissue sarcomas among pediatric patients[33,34] and 10% among adults[35,36].

The secondary objectives of this study are to further evaluate the safety and tolerability of this regimen, estimate progression-free survival (PFS) and overall survival (OS).



Legend

-- Gemcitabine 900 mg/m2 given at a fixed dose rate of 10 mg/m^2/min on D1, D8 and D15 to be given over 90 min every 28 days

-- IV Ascorbate 75gm will be given on days 1, 2, 8,9,15 & 16

-- Patient will be assessed every 2 cycles for progression. Treatment will continue for at least 6 cycles if no disease progression. Treatment can continue with either concomitant ascorbate or gemcitabine or single agent gemcitabine beyond 6 cycles as per investigator

--Treatment will be terminated with progression of disease

4. STUDY SUBJECTS

4.1 Subject Selection

All patients will be obtained from the University of Iowa cancer center patient population. Patients must have baseline evaluations performed prior to the first dose of study drug and must meet all inclusion and exclusion criteria. Results of all baseline evaluations, which assure that all inclusion and exclusion criteria have been satisfied, must be reviewed by the Principal Investigator or sub investigator prior to enrollment of that patient. In addition, the patient must be thoroughly informed about all aspects of the study, including the study visit schedule and required evaluations and all regulatory requirements for informed consent. The written informed consent must be obtained from

the patient prior to enrollment. The following criteria apply to all patients enrolled onto the study unless otherwise specified.

4.2 Inclusion Criteria

1. Male or female patients aged ≥ 18 years old
2. ECOG Performance Status of ≤ 2
3. Ability to provide written informed consent obtained prior to participation in the study and any related procedures being performed
   - Patients must meet the following laboratory criteria:
   - Hematology:
     - Neutrophil count of >1500/mm^3
     - Platelet count of > 100,000/mm^3L
     - Hemoglobin ≥ 9 g/dL (transfusion to meet eligibility allowed)
   - Biochemistry:
     - AST/SGOT and ALT/SGPT ≤ 2.5 x upper limit of normal (ULN) or ≤ 5.0 x ULN if the transaminase elevation is due to disease involvement
     - Alkaline phosphatase < 5 x ULN
     - Serum bilirubin ≤ 1.5 x ULN
     - Serum creatinine ≤ 1.5 x ULN or 24-hour creatinine clearance ≥ 50 ml/min
     - Total serum calcium ≥ LLN or if calcium is below LLN then corrected calcium for serum albumin should be >/= LLN
     - Serum potassium ≥ LLN
     - Serum sodium ≥ LLN
     - Serum albumin ≥ 3g/dl
4. Tolerate a 15g ascorbate infusion (screening dose)
5. Baseline MUGA or ECHO done only in subjects with prior doxorubicin exposure. The test must demonstrate LVEF ≥ the lower limit of the institutional normal.
6. Women of childbearing potential (WOCBP) must have a negative serum pregnancy test within 7 days of the first administration of study treatment and must be willing to use two methods of contraception one of them being a barrier method during the study and for 3 months after last study drug administration
7. Any patient with the diagnosis of locally advanced, unresectable or metastatic soft tissue or bone sarcoma (except GIST and Kaposi's) from any site. A minimum of 1 prior chemotherapy regimen, including adjuvant or neo-adjuvant therapy for the treatment of sarcoma. Patients eligible for an anthracycline should have received a prior anthracycline containing regimen. Patients who decline or are not eligible for anthracycline treatment may be considered for this protocol as a first line treatment. Patients with a diagnosis of liposarcoma should also have received eribulin if they received anthracycline-based therapy prior to eribulin. Patients with a diagnosis of myxoid liposarcoma should have received trabectedin. Patients with angiosarcoma

should have received either taxol or docetaxel. Patients must have measurable disease defined as at least 1 lesion ≥ 1cm in the greatest dimension.

8. Patients with metastatic bone sarcomas who have failed all available therapies that have demonstrated clinical benefit. Available therapies include but not limited to methotrexate, adriamycin and cisplatin for osteosarcoma and vincristine, adriamycin and Cytoxan, ifosfamide, etoposide (VAC/IE)for Ewing's sarcoma.
9. Previous exposure to Gemcitabine will only be allowed if there is no residual toxicity from previous treatments. Toxicity must be graded as 0 or 1 prior to study.
10. Patients *must* have had disease progression on or following their most recent treatment regimen or on presentation for the first time with locally advanced unresectable or metastatic disease.

4.3 **Exclusion Criteria**

1. G6PD (glucose-6-phosphate dehydrogenase) deficiency
2. New York Heart Association (NYHA) Grade II or greater congestive heart failure (see Appendix E)
3. History of myocardial infarction or unstable angina within 6 months prior to Day 1
4. History of stroke or transient ischemic attack within 6 months prior to Day 1
5. Known CNS disease, except for treated brain metastasis: Treated brain metastases are defined as having no evidence of progression or hemorrhage after treatment and no ongoing requirement for dexamethasone, as ascertained by clinical examination and brain imaging (MRI or CT) during the screening period. Anticonvulsants (stable dose) are allowed. Treatment for brain metastases may include whole brain radiotherapy (WBRT), radiosurgery (RS; Gamma Knife, LINAC, or equivalent) or a combination as deemed appropriate by the treating physician. Patients with CNS metastases treated by neurosurgical resection or brain biopsy performed within 3 months prior to Day 1 will be excluded
6. Actively receiving insulin or requiring fingerstick glucose monitoring at time of ascorbate infusion (unless an exception is granted by the IND sponsor, medical monitor, and the PI).
7. Significant vascular disease (e.g., aortic aneurysm, requiring surgical repair or recent peripheral arterial thrombosis) within 6 months prior to Day 1
8. Evidence of bleeding diathesis or significant coagulopathy (in the absence of therapeutic anticoagulation)
9. Pregnancy (positive pregnancy test) or lactation. Use of effective means of contraception (men and women) in subjects of child-bearing potential
10. Patients who are on the following drugs and cannot have a drug substitution: flecainide, methadone, amphetamines, quinidine, and chlorpropamide. High dose ascorbic acid may affect urine acidification and, as a result, may affect clearance rates of these drugs.
11. Other concurrent severe and/or uncontrolled medical conditions
12. Patients who have received chemotherapy or any investigational drug < 2 weeks prior to starting study drug or who have not recovered from side effects of such therapy.

13. Major surgical procedure, open biopsy, or significant traumatic injury within 28 days prior to Day 1 or anticipation of need for major surgical procedure during the course of the study.
14. Concomitant use of any other anti-cancer therapy or radiation therapy. Palliative radiation therapy to non-target lesions is permitted.
15. Male patients whose sexual partners are WOCBP not using a double method of contraception during the study and 3 months after the end of treatment. One of these methods must be a condom.
16. Patients with a history of another primary malignancy within 2 years other than curatively treated CIS of the cervix, or basal or squamous cell carcinoma of the skin
17. Patients with known positivity for human immunodeficiency virus (HIV); baseline testing for HIV is not required. High-dose ascorbate acid is a known CYP450 3A4 inducer, which results in lower serum levels of antiretroviral drugs.[37]
18. Patients with any significant history of non-compliance to medical regimens or with inability to grant a reliable informed consent
19. Patients with GIST tumors and Kaposi's Sarcoma are excluded
20. Patients with history of more than one symptomatic oxalate stone in the last 6 months or visible stone in the kidney or ureter on screening CT scan.

5. TREATMENT PLAN (SEE SECTION 3 FOR OVERALL STUDY DESIGN AND SCHEMA)

Once informed consent is obtained:

- Enter subject into the OnCore database of the Holden Comprehensive Cancer Center for DSMC notification.
- Scan a copy of-signed eligibility criteria into OnCore.
-
- Scan the G12 into the subject's medical record.

5.1 Post-consent Screening Procedures

If subjects fail the G6PD or test doses of ascorbate, they are considered a screen failure.

5.1.1 **G6PD test** (off-site test, so draw immediately). If subject has a prior G6PD test, this can be used as G6PD levels do not fluctuate (i.e., there is no window for this test).

5.1.2 **Ascorbate initiation** (one test dose of ascorbate): Can be given any time through the day prior to chemotherapy with one 15-g infusion over 30 minutes. Failure of the 15g test dose is defined as any toxicity ≥ CTCAE grade 3 or a significant medical event (in the opinion of the principal investigators).

5.1.3 **Concomitant Ascorbate (High-dose Ascorbic Acid) Infusions**

5.1.3.1 **Participant dose**. After completing a 15g test dose for screening, subjects will receive a 75g dose. Dose modifications are not made for weight or body surface area.

5.1.3.2 **Administration**. Based on subject tolerance; infusion rate should not exceed 500 mL/hour without consulting with physician. Recommended infusions times are provided (Table 1) but may be adjusted for subject comfort. Changes in infusion rates should be recorded.

5.1.3.3 **Order of therapy:** Ascorbate is infused prior to gemcitabine

5.1.3.4 **Schedule**. Schedule two times weekly, must be on separate calendar days. (within 7 sequential days; does not have to be a calendar week)

5.1.3.5 **Cycle**. Concomitant with gemcitabine, 28 day cycle.

5.1.3.6 **Duration.** Subjects will continue with therapy until one of the criterions for removal is met (Section 5.5). Patients with complete response, partial response or stable disease will continue for two more cycles of gemcitabine, ascorbate and another CT will be done to assess response after every 2 cycles until 6 cycles. For treatment beyond 6 cycles, see section 5.3.

Table 1. Recommended Infusion Times for Pharmacological Ascorbate †

Ascorbate dose (500 mg / mL)	Total volume*	Osmolarity (mOsm/L)§	Infusion Time
15g (30 mL)	250 mL	681	30 minutes
75g (150 mL)	1000 mL	851.7	120 minutes

† Provided by Drug Information Center at the University of Iowa, July 2011

* Sterile water for injection only. Do not use D5W

§ Theoretical calculations provided by Drug Information Center; targeted osmolarity range is 500 – 900 mOsm/L

5.1.4 **Chemotherapy (Gemcitabine)**

Gemcitabine is administered following standard fixed dose infusion practice adopted at UIHC. See package insert for more details.

5.1.4.1 **Initiation**. Within 21 days of signing the consent form

5.1.4.2 **Premedications**. UIHC standard of care premedication procedures should be followed for chemotherapy administration. Thirty minutes before administering gemcitabine:

- dexamethasone 12 mg orally
- antiemetic Prochlorperazine 10 mg oral

5.1.4.3 **Dose**. 900 mg/m^2 (actual body weight) over 90 mins continuous infusion.

5.1.4.4 **Antiemetics**. Use Zofran (ondansetron) with caution as this medication may interact with high-dose ascorbic acid, resulting in sub-efficacious levels of Zofran.

5.1.4.5 **Order of therapy**. Ascorbate is infused prior to Gemcitabine

5.1.4.6 **Duration of therapy** – Up to 6 cycles. Subjects will continue with therapy until one of the criterions for removal is met (Section 5.5). Subjects with complete response, partial response or stable disease will continue for two more cycles of gemcitabine, ascorbate and another CT will be done to assess response after every 2 cycles until 6 cycles. For treatment beyond 6 cycles, see section 5.3.

5.1.4.6 **Dose modifications.** Provided in Section 7.2.5

5.2 **General Concomitant Medication and Supportive Care Guidelines**

5.2.1 **Prophylactic antibiotics for neutropenia**: To be considered by the treating physician for neutropenia. Ciprofloxacin 500 orally, twice daily, is encouraged if ANC < 500/mm^3

5.2.2 **Imaging Contrast.** Iodine based imaging contrast should not be administered on the same day with ascorbate infusion. If administered within the same day, monitor liver function tests and contact the medical monitor for the IND.

5.2.3 **CYP450 3A4 interactions**. Review drugs for the potential for interactions. Medically monitor subjects receiving a potentially interactive drug.

5.2.4 **Hyperuricemia** (gout)**.** Initial dose of colchicine should be 0.6 mg with a subsequent dose of 0.3 mg an hour later if the first dose is well-tolerated. Liver function tests (AST / ALT) should be monitored.

5.2.5 **Hypertension.** Subjects should have blood pressure monitored at the start of each cycle. For subjects with documented hypertension, treating physicians should consider subsequent monitoring. Hypertensive medication should be initiated or increased for optimal blood pressure control according to standard public health guidelines.

5.2.6 **Fluid intake.** Subjects will be encouraged to maintain adequate hydration to decrease the risk of nephrolithiasis. Those unable to maintain oral hydration should be considered for supplemental IV hydration per institutional care guidelines.

5.2.7 **G-CSF products**. The use of G-CSF products is allowed on the study per the treating physician's discretion.

5.3 **Duration of Therapy Beyond 6 Cycles**

In the absence of treatment delays due to adverse event(s), treatment may continue until criteria for removal is met (Section 5.4.2and 5.5). If there are delays due to adverse events, continuation of the protocol and continued treatment will be determined by the treating medical oncologist and IND sponsor. Subjects whose disease has not progressed while receiving gemcitabine and ascorbate and who are tolerating therapy well may continue concomitant treatment with gemcitabine and ascorbate on trial beyond 6 cycles. The disease assessment will occur every 2 cycles. The investigator may choose to continue single agent gemcitabine off trial. Subject will be followed for survival for up to 2 years. Single agent ascorbate beyond 6 cycles will not be allowed. The ascorbate level and the reactive oxygen species research blood draw levels need not be drawn beyond 6 cycles of concomitant therapy.

5.4 **Duration of Follow Up**

5.4.1 **Early termination.** Subjects who withdraw or were removed from study prior to completing all 6 cycles of concomitant therapy will be followed a minimum of 21 days from the final ascorbate infusion. Further follow-up is per investigator discretion.

5.4.2 **Completed therapy.** Subjects who complete all six cycles of concomitant therapy will be actively followed for 2 years from the end of study-directed therapy. Follow-up assessments are listed in Section 6.3

5.4.3 The first follow-up appointment will be 21 days (+/- 10 days) after the last infusion of study drug. Follow-up appointments should be scheduled every three months thereafter (±2) calendar week). The final study appointment will be the subject's 2 year follow-up appointment (± 1 month). Subjects will be passively followed through chart review for progressive free survival and overall survival.

5.5 Interruption or Discontinuation of Treatment

For patients who are unable to tolerate the protocol-specified dosing schedule, dose adjustments are permitted in order to keep the patient on study drug. If administration of any drug must be interrupted because of unacceptable toxicity, drug dosing will be interrupted or modified according to rules described in Section 7.2.5. Toxicity will be assessed using the NIH-NCI Common Terminology Criteria for Adverse Events, version 4.0 (CTCAE v4.0). All interruption or changes to study drug administration must be recorded.

It will be documented whether or not each patient completed the clinical study. If for any patient either study treatment or observations were discontinued, the reason will be recorded.

Reasons that a patient may discontinue treatment are considered to constitute one of the following:

1. Subject's condition no longer requires study treatment (e.g. satisfactory therapeutic response)
2. Disease progression
3. Adverse event(s)
4. Abnormal laboratory value(s)
5. Abnormal test procedure result(s)
6. Protocol violation
7. Subject withdrew consent
8. Lost to follow-up
9. New cancer therapy
10. Death
11. Grade ≥ 2 pulmonary or CNS hemorrhage; any Grade 4 hemorrhage
12. Unwillingness or inability of subject to comply with study requirements
13. Determination by the investigator that it is no longer safe for the subject to continue therapy

5.6 Toxicities and Delays

Patients whose treatment is interrupted or permanently discontinued due to an adverse event or abnormal laboratory value must be followed until resolution or stabilization of the event, whichever comes first.

If a patient requires a dose delay of > 21 days from the intended day of the next scheduled dose, then the patient must be discontinued from the study. If however the patient was clearly benefiting from this therapy, the patient may be able to continue treatment with a dose reduction at the Investigator discretion, after resolution of the adverse event. All patients will be followed for adverse events and serious adverse events for at least 4 weeks following the last dose of any drug. Collection of new AEs will end if a new treatment regimen is initiated prior to the EOT visit. Patients will be followed every 3 months for disease status, new or ongoing therapies and survival status until death. This information can be obtained through a clinic visit, phone call or email.

6. VISIT SCHEDULE AND ASSESSMENTS

6.1 Visit Schedule

Required Data	Screening (28 day window up to day 1 of treatment)	Day 1 of each cycle[11]	Day 2 of each cycle	Day 8 of each cycle	Day 9 of each cycle	Day 15 of each cycle	Day 16 of each cycle	End of treatment 21 days from last treatment(+/- 7days)	Follow up (every 3 months +/- 2wks)x 2 years
Progress Note	X	X		X[12]				X	X
Examination	X	X						X	X
Vital signs[1]	X	X	X	X	X	X	X	X	X
Ht[2] and Wt	X	X		X				X	
BSA		X							
Performance status	X	X						X	X
Drug Toxicity Assessment	X	X		X		X		X	
CBC, differential	X	X		X		X		X	X[13]
G6PD[3]	X								
Chemistries[4]	X	X		X		X		X	X[13]
Glucose	X	X							
Serum pregnancy[5]	X								
UA	X								
LDH	X							X	
Ascorbate level[6]		X	X						
Blood for ROS[7]	X		X						X[13]
Gemcitabine		X[15]		X[15]		X[15]			
Ascorbate infusion	Test dose	X	X	X	X	X	X		
CT Chest, Abdomen, Pelvis[8] or MRI of lesion[9]	X	Every 2 cycles						X	X[14]
EKG; MUGA/ Echo[10]	X								

1. T,P,R,B/P. Blood pressure should be obtained before and after (≤ 15 min EOI) ascorbate infusion

2. Ht will only be taken once

3. G6PD may be obtained at any time prior to test dose as the value does not fluctuate

4. Chemistries listed in section 6.5.5 in protocol

5. Serum pregnancy test will be done in all females of child bearing potential; see section 6.5.6

6. Draw blood specimens ~4cc green top tube Day 1 pre- and Day 2 post- infusion (at 5min and 60 min post) drawn once per odd cycles. Should be stopped after 6 cycles of therapy.

7. Draw 1 EDTA pink-top tube (5-6 mL) prior to 15 g test dose, D2 post-infusion and put on ice for transport. Should be stopped after 6 cycles of therapy except for one collection at first follow up visit.

8. CT of the chest, abdomen and pelvis with contrast will be done at screening (obtained ≤ 30d from D1) obtain every 2 cycles (approximately 21 – 28 days after D1). DO NOT schedule on days with ascorbate infusions. Ascorbate and contrast are contra-indicated.

9. If limb primary, subject will need MRI of the lesion; if abdominal/pelvic lesion, a CT scan will suffice.

10. MUGA or Echo only obtained if subject has had prior exposure to doxorubicin.

11. Screening labs are valid up to 7 days prior to day 1 of the treatment cycle

12. Cycle 1 only

13. Only collected at follow up #1

14. CT scan as clinically indicated.

15. +/- 1 day allowed for gemcitabine infusion

Pre-infusion is defined as any time during the same calendar day prior to infusion.

BSA calculated at day 1 of each cycle and dose changed for 10% weight change

6.2 Efficacy Assessments

6.2.1 Tumor Response

Tumor response will be defined using the RECIST 1.1 guidelines as below

- Complete response is the disappearance of all target lesions;
- Partial response is a 30% decrease in the sum of the longest dimension (LD) of target lesions, relative to baseline measurement;
- Progressive disease is an increase of 20% or more in the sum of the LD of target lesions;
- Stable disease is a decrease in tumor size of less than 30% or increase of less than 20%.

6.2.2 Progression Free Survival

Time from start of therapy (day 1, cycle 1) to documented disease progression or death due to any cause. Progression will be defined using the RECIST 1.1

guidelines.[38]

6.2.3 Overall Survival

Time from start of therapy (day 1, cycle 1) to death.

6.2.4 Toxicity

Categorize and quantify adverse events from start of therapy (day 1, cycle 1) to end of study

6.3 **Follow up assessments**

The following assessments should be done at follow-up visits after completion of protocol-directed therapy. The first scheduled follow up is 21 days after the last ascorbate infusion (+/- 7 days).

6.3.1 (follow-up #1, only) Blood sample for ROS testing. Draw 5-6 cc in a pink-top tube and put on ice for transport.

6.3.2 (follow-up #1, only) CBC w/differential

6.3.3 (follow-up #1, only) Comprehensive metabolic panel.

6.3.4 If labs were ordered for another clinic visit, and within window, these can be used at the discretion of the medical monitor, sponsor, and investigator.

6.3.5 Lab information, including CBC w/differential and plasma chemistries, ordered for standard of care or other reasons may be pulled from subsequent follow-up visits or physician's visits, for the purposes of monitoring treatment effects and lingering adverse events .

6.3.6 Medical record information will be reviewed for the purposes of monitoring treatment effects and lingering adverse events.

6.3.7 Follow up CT scans as clinically indicated for standard of care

6.4 **End of Treatment Assessments**

The following assessments should be performed when a criteria for removal is met (Section 5.5). An End-of-Treatment (EOT) visit should be scheduled within 21days (+/-7 days)from last study drug infusion. An existing clinical visit may be used for the EOT visit.

6.4.1 **Minimum follow-up.** Every effort will be made to follow patients who come off treatment for toxicity per the schedule of events. If the subject does not return to clinic, phone call contact is acceptable. If phone call contact cannot be made, and the subject does not return a phone call, documentation should be made of at least 3 attempts.

6.5 **Safety Assessments**

Safety assessments will consist of monitoring and recording all adverse events and serious adverse events, the regular monitoring of hematology, blood chemistry and urine values, vital signs, ECOG performance status, and the regular physical examinations.

Adverse events will be assessed according to the Common Toxicity Criteria for Adverse Events (CTCAE) version 4.03. CTCAE v4.0 can be accessed on the NIH/NCI website at http://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03_2010-06-14_QuickReference_5x7.pdf

6.5.1 **Adverse events**

Information about all adverse events, whether volunteered by the subject, discovered by investigator questioning, or detected through physical examination, laboratory test or other means, will be collected and recorded and followed as appropriate. Adverse events will be collected for 30 days post last dose of drug, or until new therapy is initiated, whichever occurs first

An adverse event is the appearance or worsening of any undesirable sign, symptom, or medical condition occurring after starting the study drug even if the event is not considered to be related to study drug. Medical conditions/diseases present before starting study drug are only considered adverse events if they worsen after starting study drug. All patients will be followed for adverse events and serious adverse events for at least 4 weeks following the last dose of study drug.. (For Adverse Event Reporting: see Section 9.0 Safety Monitoring)

The occurrence of adverse events should be sought by non-directive questioning of the patient at each visit during the study. Adverse events also may be detected when they are volunteered by the patient during or between visits or through physical examination, laboratory tests (deemed clinically significant or treated), or other assessments. As far as possible, each adverse event should be evaluated to determine:

1. the severity grade (mild, moderate, severe) or (grade 1-4)
2. its relationship to the study drug(s) (suspected/not suspected)
3. its duration (start and end dates or if continuing at final exam)
4. action taken (no action taken; study drug dosage adjusted/temporarily interrupted; study drug permanently discontinued due to this adverse event; concomitant medication taken; non-drug therapy given; hospitalization/prolonged hospitalization)
5. whether it constitutes a serious adverse event (SAE)

All adverse events should be treated appropriately. Such treatment may include changes in study drug treatment including possible interruption or discontinuation, starting or stopping concomitant treatments, changes in the frequency or nature of assessments, hospitalization, or any other medically required intervention. Once an adverse event is detected, it should be followed until its resolution (or stabilized), and assessment should be made at each visit (or more frequently, if necessary) of any changes in severity, the suspected relationship to the study drug, the interventions required to treat it, and the outcome.

Information about common side effects already known about the investigational drug can be found in the [Investigators' Brochure] or will be communicated between IB updates in the form of Investigator Notifications. This information will be included in the patient informed consent and should be discussed with the patient during the study as needed.

6.5.2 **Serious Adverse Events**

Information about all serious adverse events will be collected and recorded. To ensure patient safety each serious adverse event must also be reported to data and safety monitoring committee (DSMC) within 24 hours of learning of its occurrence, see section 9.3 for details of reporting. A serious adverse event is an undesirable sign, symptom or medical condition which:

- is fatal or life-threatening
- results in persistent or significant disability/incapacity
- constitutes a congenital anomaly/birth defect
- requires inpatient hospitalization or prolongation of existing hospitalization, unless hospitalization is for:
    - routine treatment or monitoring of the studied indication, not associated with any deterioration in condition (specify what this includes)
    - elective or pre-planned treatment for a pre-existing condition that is unrelated to the indication under study and has not worsened since the start of study drug
    - treatment on an emergency outpatient basis for an event not fulfilling any of the definitions of a SAE given above and not resulting in hospital admission
    - social reasons and respite care in the absence of any deterioration in the patient's general condition is medically significant, i.e., defined as an event that jeopardizes the patient or may require medical or surgical intervention to prevent one of the outcomes listed above.

6.5.3 **Vital Signs**

Vital sign assessment consists of height (first visit), pulse, blood pressure, respiration rate, temperature and weight. . Blood pressure should be obtained before and after (≤ 15 min EOI) ascorbate infusion. If the infusion runs for longer than 1 hour, blood pressure assessment should be done in the interm as clinically indicated.

Pre-infusion is defined as any time during the same calendar day prior to infusion.

Post infusion is ≤ 15 minutes from end of infusion

6.5.4 **Physical Examination**

Physical examination will be performed which must comprise a total body examination (general appearance, skin, neck, including thyroid, eyes, ears, nose, throat, lungs, heart, abdomen, back, lymph nodes, extremities and basic nervous system).
Significant findings made after the start of study drug which meet the definition of an Adverse Event must be recorded.

6.5.5 **Laboratory Evaluations**

Laboratory evaluation should be done at baseline (within ≤ 7 days prior to dosing prior to the first administration of ascorbate test dose), during the course of the study and at the time of the study treatment completion visit. Results must be reviewed prior to administering any agent. More frequent examinations may be performed if medically indicated; results should be recorded.

Hematology

Hematology must include hemoglobin, hematocrit, platelets, total white blood cell count (WBC) and differential.

Blood Chemistry

Biochemistry includes the following parameters: BUN, creatinine, calcium, sodium, potassium, chloride, CO_2 (HCO_3), total bilirubin, alkaline phosphatase, AST/SGOT, ALT/SGPT, GGT. If total bilirubin is greater than the upper limit of normal, direct and indirect bilirubin should be performed. Albumin level needed at baseline only

G6PD levels

Glucose – 6 phosphate dehydrogenase levels will be checked at screening visit. Pt will be excluded from the study if he is found to be deficient.

Urinalysis

Standard urinalysis assessment (pH, protein, glucose, blood, ketones, and leukocytes) can be performed. This must be supplemented with laboratory quantification of any potentially relevant abnormalities

6.5.6 Serum Pregnancy Test

All females of childbearing potential should complete a serum pregnancy test within 7 days prior to the administration of the protocol i.e. prior to cycle 1 day 1. Postmenopausal women must have been amenorrheic for ≥ 12 months in order to be considered "of non-childbearing potential". The subjects in this study are being treated for stage IV sarcoma, and are unlikely to become pregnant. The PI will discuss, before the study, the importance of contraceptives with woman of child bearing age. If he considers a subject at risk for become pregnant, he will order a pregnancy test during any clinic visit the need presents itself.

6.5.7 Performance Status

Performance status will be assessed

ECOG PERFORMANCE STATUS*	
Grade	**ECOG**
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	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	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair
5	Dead

* As published in Am. J. Clin. Oncol.:
Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And

Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.

7. STUDY MEDICATIONS

7.1 Ascorbic Acid Injection, USP; Ascorbate; NDC 67457-118-50

7.1.1 **Availability**. Ascorbic Acid Injection, USP (Bioniche Pharma USA LLC) is a commercially available injection available in sterile single-use 50 mL vials containing 25 g of ascorbic acid (500 mg/mL).

7.1.2 **Compatibility.** Ascorbate is considered a CYP4503A4 inducer.32 Therefore close monitoring of subjects who may be concomitantly receiving CYP3A4 substrates with narrow therapeutic indexes for toxicities is required. A table of CYP3A4 substrates, inhibitors, and inducers is provided in the Appendix. Ascorbate may decrease plasma/blood concentrations of **substrates** (Appendix B, tables 1, 2, and 3). Ascorbate may increase CYP3A4 induction (Appendix B, Table 4). Imaging contrast should not be given on the same day as an ascorbate infusion.

7.1.3 **Storage and Stability**. Unopened vials of Ascorbic Acid for Injection USP are stable until the expiration date indicated on the package when stored between 2° – 8°C (36°–46°F). Protect from light and store in the carton until time of use.

7.1.4 **Toxicities**.

Diarrhea, nausea and/or vomiting, kidney stones, dry mouth/thirst, headache, abdominal pain, fatigue, facial flushing, sweating, weakness, injection site irritation, and faint/dizzy (after rapid infusion) have been reported in the literature. When administered on the same day as imaging contrast, liver function tests may be elevated. Recommendation is to not schedule imaging with contrast on the same day as an ascorbate infusion.

7.1.5 **Ascorbate Dose modifications/delays.**

There are no dose adjustments for toxicities. If an unexpected adverse event is observed, the treating physicians may withhold ascorbic acid for up to 1 calendar weeks to determine if the effect diminishes or resolves entirely. Considering the nature and severity of the event, if reasonable ascorbic acid should then be continued to determine if the event again presents. This will function as a test for causality. Due to holidays or inclement weather, doses may be delayed by up to one week with physician and PI approval. Reason for treatment delay should be noted in the study chart. Missed doses should not be made up. If holding Gemcitabine, then Ascorbate as single agent can be given as per discretion of investigator.

7.2 Gemcitabine

Gemzar® (gemcitabine HCl) is a nucleoside analogue that exhibits antitumor activity. Gemcitabine HCl is 2′-deoxy-2′,2′-difluorocytidine monohydrochloride (β -isomer). The structural formula is as follows: The empirical formula for gemcitabine HCl is $C_9H_{11}F_2N_3O_4$•HCl. It has a molecular weight of 299.66.

7.2.1 **Availability**

Gemcitabine HCl is a white to off-white solid. It is soluble in water, slightly soluble in methanol, and practically insoluble in ethanol and polar organic solvents.

The clinical formulation is supplied in a sterile form for intravenous use only. Vials of Gemzar contain either 200 mg or 1 g of gemcitabine HCl (expressed as free base) formulated with mannitol (200 mg or 1 g, respectively) and sodium acetate (12.5 mg or 62.5 mg, respectively) as a sterile lyophilized powder. Hydrochloric acid and/or sodium hydroxide may have been added for pH adjustment.

7.2.2 **Compatibility**. Compatibility with other drugs has not been studied. No incompatibilities have been observed with infusion bottles or polyvinyl chloride bags and administration sets

7.2.3 **Storage and stability**

Store at controlled room temperature 20° to 25°C (68° to 77°F). The USP has defined controlled room temperature as "A temperature maintained thermostatically that encompasses the usual and customary working environment of 20° to 25°C (68° to 77°F); that results in a mean kinetic temperature calculated to be not more than 25°C; and that allows for excursions between 15° and 30°C (59° and 86°F) that are experienced in pharmacies, hospitals, and warehouses."

7.2.4 **Toxicities**

Gemzar® can suppress bone marrow function as manifested by leukopenia, thrombocytopenia, and anemia and myelosuppression is usually the dose-limiting toxicity. Pulmonary toxicity has been reported. Hemolytic Uremic Syndrome (HUS) and/or renal failure have been reported following one or more doses. Renal failure leading to death or requiring dialysis, despite discontinuation of therapy, has been reported. The majority of the cases of renal failure leading to death were due to HUS. Serious hepatotoxicity, including liver failure and death, has been reported in patients receiving Gemzar® alone or in combination with other potentially hepatotoxic drugs

7.2.5 **Dose Modifications for Gemcitabine**

7.2.5.1 **Hematologic toxicity**

Dosage adjustment is based upon the degree of hematologic toxicity experienced by the patient on the day of dosing as well as those in the prior cycles (see 7.2.5.2). Patients must have an ANC of >1000 and a platelet count of >100,000 on Day 1 of each cycle to begin treatment with gemcitabine. On Day 8 and 15 of the chemotherapy cycle the below guidelines will be used.

Absolute granulocyte count (x 10^6/L)		**Platelet count (x 10^6/L)**	**% of full dose**
≥ 1000	And	≥ 100,000	100 (dose level 0)
500-999	Or	50,000-99,999	75 (dose level -1)
< 500	Or	< 50,000	Hold

Missed doses of gemcitabine are not made up.

Dose levels of gemcitabine as below:

Dose level 0	900 mg/m2 over 90 min
Dose level -1	675 mg/m2 over 67.5 min
Dose level -2	500/m2 over 50 mins

Neutropenic fever.

If a subject experiences grade 3 or 4 neutropenia associated with a febrile event (temperature > 38.3°C) at any time, the gemcitabine dose will be held until ANC > or = 1000/mm^3. When restarted, gemcitabine will be reduced 1 dose level. This dose reduction applies to the remainder of treatment. Use of growth factor support is permitted as per discretion of the treating physician.

7.2.5.2 **Thrombocytopenia, bleeding.**
If a subject experiences a clinically significant bleeding episode and/or platelet nadir of < 25,000/mm^3 at any time during therapy, further gemcitabine doses will be held until platelets > 25,000 and bleeding resolves (see table above). When restarted, gemcitabine will be reduced one dose level. This dose reduction applies to the remainder of combination therapy. [significant bleeding episode is defined as one requiring a transfusion or other medical intervention to stop the bleeding

7.2.6 **Non hematologic toxicity**
Nausea and vomiting should be treated aggressively with standard antiemetics. If still uncontrolled, follow the table below. This applies to all non-hematologic toxicities. Dose reductions for **non- hematologic toxicities** with Gemcitabine will be permanent with the following exceptions. The investigator will be allowed to dose modify at their discretion for minor AEs or AEs that may be corrected by other measures. Examples of minor non-hematological toxicities include alopecia, nausea, vomiting, fever without grade IV neutropenia, or metabolic imbalances that may be corrected by provision of supportive care (e.g. hypokalemia and provision of potassium). For minor AEs in which the patient recovers, the investigator will be allowed to re-escalate.

Toxicity grade	Gemcitabine	Ascorbate
0–2 (& grade 3 nausea/ vomiting)	Continue dose	Continue treatment
3	Decrease one dose level	Continue per discretion of investigators
4	Hold until toxicity resolves to ≤ grade 2 (≤ grade 3 for nausea & vomiting). Decrease 1 dose level for gemcitabine when resuming concurrent ascorbate. This dose reduction applies to the remainder of concurrent treatment.	

7.2.6.1 **Gemcitabine Dose Modifications for Hepatic Toxicity.** The following dose adjustments should be made for patients experiencing hepatic toxicity at any time during treatment. Hepatic toxicity should be graded using Common Terminology Criteria for Adverse Events v4.0 (CTCAE).

- When held for liver toxicity, gemcitabine will be resumed at one dose reduction when the toxicity resolves to grade 2 or less. This will be maintained for the remainder of combination therapy.

Hepatic toxicity (AST or ALT or Bilirubin or ALP)	**Gemcitabine dose level**
Grade 0 – 2	No change
Grade 3	Decrease one dose level*
Grade 4	Hold**
* Patients who have a repeated episode of Grade 3 hepatic toxicity after dose reduction of gemcitabine therapy should discontinue gemcitabine.	
** Patients with grade 4 hepatic toxicity should be evaluated for disease progression. If disease progression is found, discontinue protocol therapy (gemcitabine and ascorbate). If no disease is found resume gemcitabine at one dose level lower than the previous dose when toxicity resolves to grade 0 - 2. If toxicity does not resolve to grade 0 - 2 within 4 weeks, discontinue protocol therapy.	

7.2.6.2 **Gemcitabine Dose Modifications for Edema**. Edema, not associated with any evidence of cardiac, hepatic or renal failure, has been reported in patients receiving gemcitabine. Edema should be graded using Common Terminology Criteria for Adverse Events v4.0 (CTCAE). When held for edema toxicity, gemcitabine will be resumed at one dose reduction when the toxicity resolves to grade 2 or less. This will be maintained for the remainder of combination therapy.

Grade of Edema	Gemcitabine dose level
1 or 2	No change
3	Decrease one dose level

8. SAFETY MONITORING

This study will also be monitored by internal oversight specialists at the University of Iowa. *The Data and Safety Monitoring Plan of the Holden Comprehensive Cancer Center* provides standard operating procedures to monitor all clinical cancer trials at the UIHC. All investigator-initiated trials are automatically monitored by the Data and Safety Monitoring Committee (DSMC). A detailed data and safety monitoring plan for this study is provided (Appendix C). This study has been assigned as a **risk level 4** as a physician sponsored IND phase study.

8.1 Determination of Reporting Requirements

An adverse event (AE) is defined in the *CTEP, NCI Guidelines* [2005] as "any unfavorable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of a medical treatment or procedure (attribution of unrelated, unlikely, possible, probably or definite)."

8.2 **Adverse event recording.** The clinical research team is responsible for identifying, collecting, and recording clinical data, including adverse events, through 30 days after the last dose of study drug (ascorbate).

8.3 **Adverse event grading.** Adverse events will be graded according to NCI's Common Toxicity Criteria (CTCAE v4). Grading may be done by any licensed medical personnel (e.g., research nurse, treating physician, etc.) with final determination made by the principal investigator.

8.4 **Adverse event attribution.** Initial attribution of ascorbate to a grade 3 or greater event may be assigned by any licensed medical personnel (e.g., research nurse, treating physician, etc.). The principal investigator will make the final determination regarding the attribution of the study drug to the adverse event and also decide course of action for the study participant. CTCAE grade 2 and lesser events do not require attribution *unless* the event is unexpected (i.e., not related to chemotherapy, tumor, or previously diagnosed underlying condition).

8.5 Routine Adverse Event Reporting Requirements

Adverse events attributed to the study drug will be reported to the Data Safety Monitoring Committee (DSMC) *via* the Clinical Research Safety Officer (CRSO) following established standard operating procedure of the DSMC.

8.6 **Expedited Adverse Event Reporting**

Investigators MUST immediately report to the DSMC any serious adverse events (SAE), whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64). Reporting requirements are in Appendix C.

9. CORRELATIVE/SPECIAL STUDIES

Parameters of oxidative stress. Research blood for ROS and ascorbate testing will be obtained at each cycle according to the table below:

•

The last ROS blood sample will be obtained at the first follow-up visit (protocol section 6.5 Follow-up Assessments) prior to any therapy that would be administered that day.

ROS research samples are. 5-6 mL in an EDTA tube (pink top) and placed immediately on ice. The basic science RA must be contacted immediately after the blood draw is obtained. Samples should be spun to yield plasma using standard procedures as soon as possible. Plasma should then be transferred to a cryovial and frozen immediately at -80°C. It is imperative that plasma is not thawed until analysis in the Eicosanoid Core Laboratory as F_2-IsoPs can be generated in plasma *ex vivo*, even at temperatures of -20°C. A minimum of 1 mL plasma is required for analysis. This research analysis will not be added to the subject's medical record.

Time point	ROS (pink*) pre dose	ROS (pink*) post dose	Ascorbate level (green top) pre dose odd cycles	Ascorbate level (green top) post dose 5 min & 60 min odd cycles
Test Dose	x			
C1D1			x	
C1D2		x		x
C2D1				
C2D2		x		
C3D1			x	
C3D2		x		x
C4D1				
C4D2		x		
C5D1			x	
C5D2		x		x
C6D1				

C6D2		x		
Follow up #1	x			

*Pink tubes go on ice

Ascorbate level: Draw 4cc green top (sodium heparin) tube. Keep ambient.

ROS sample: Draw in a 5-6 cc pink top (EDTA) tube. Put on ice for transport.

For sample pick up email: RadOncLabSamples@healthcare.uiowa.edu

10. STATISTICAL CONSIDERATIONS

10.1 Endpoints

- Tumor response
- Progression free survival (PFS)
- Overall survival (OS)
  - Safety profile

10.2 Sample Size & Accrual

A response rate of 10% or less is considered to be clinically uninteresting, whereas a response rate of at least 30% may warrant further investigation. Sample size requirements are based on an optimal Simon two-stage design with 80% power and a significance level of 5%. Ten (10) patients will be enrolled in the first stage, and the study will be terminated if 1 or fewer respond. Otherwise an additional 19 patients will be enrolled in the second stage. If 6 or more of the 29 total patients respond, the treatment will be deemed worthy of further investigation.

A total of 29 evaluable patients will be enrolled in this study. The anticipated accrual rate is 2 patients per month who would meet inclusion criteria.

10.3 Statistical Analysis & Reporting

The number and severity of all adverse events will be summarized by simple descriptive statistics. Objective response rate (CR+PR) point estimates and 95% exact confidence intervals will be reported. For PFS (time from first day of study treatment to first documented disease progression or death due to any cause) and OS (time from first day of study treatment to death due to any cause), survival curves using the Kaplan-Meier method will be constructed. Estimates along with 95% confidence intervals will be reported.

APPENDIX A: New York Heart Association (NYHA) GUIDELINES

The Stages of Heart Failure – NYHA Classification
In order to determine the best course of therapy, physicians often assess the stage of heart failure according to the New York Heart Association (NYHA) functional classification system. This system relates symptoms to everyday activities and the patient's quality of life.

Class	**Patient Symptoms**
Class I (Mild)	No limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, or dyspnea (shortness of breath).
Class II (Mild)	Slight limitation of physical activity. Comfortable at rest, but ordinary physical activity results in fatigue, palpitation, or dyspnea.
Class III (Moderate)	Marked limitation of physical activity. Comfortable at rest, but less than ordinary activity causes fatigue, palpitation, or dyspnea.
Class IV (Severe)	Unable to carry out any physical activity without discomfort. Symptoms of cardiac insufficiency at rest. If any physical activity is undertaken, discomfort is increased.

PROTOCOL APPENDIX B: CYP3A4 INTERACTION TABLE

CYP3A4 INTERACTION TABLE (Ascorbate may decrease the plasma/blood concentrations of substrates) Drugs with ~~strikeout~~ text are antiretroviral drugs that are not allowed for use on this study. * 3A4 substrate & inhibitor; # 3A4 substrate & inducer			
abiraterone	acetaminophen	ado-trastuzumab	alfentanil
alfuzosin	aliskiren	alitretinoin	almotriptan
alprazolam	ambrisentan	amiodarone*	amitriptyline
amlodipine	amprenavir	apixaban	aprepitant*
aripiprazole	armodafinil#	artemether	asenapine
astemizole	atazanavir*	atorvastatin	avanafil
axitinib	beclomethasone	bedaquiline	benzphetamine
bexarotene	bisoprolol	boceprevir	bortezomib
bosentan#	bosutinib	brentuximab	bromazepam
bromocriptine	budesonide	buprenorphine	buspirone
busulfan	cabazitaxel	cabozantinib	caffeine
canagliflozin	carbamazepine#	cevimeline	chlordiazepoxide
chloroquine	chlorpheniramine	ciclesonide	cilostazol
cinacalcet	cisapride	citalopram	clarithromycin*
clindamycin	clobazam	clomipramine	clonazepam
clopidogrel	clorazepate	clozapine	cobicistat*
cocaine	codeine	colchicine	conivaptan*
crizotinib	cyclobenzaprine	cyclophosphamide	cyclosporine*
dantrolene	dapsone	darifenacin	~~darunavir~~
dasatinib	delavirdine*	desogestrel	dantrolene
dexamethasone#	dexlansoprazole	dextromethorphan	diazepam
diclofenac	dienogest	dihydroergotamine	diltiazem*
disopyramide	docetaxel	dofetilide	dolasetron
domperidone	donepezil	doxorubicin	dronedarone
droperidol	dutasteride	efavirenz*#	eletriptan
elvitegravir	enzalutamide	eplerenone	ergoloids
ergonovine	ergotamine	erlotinib	erythromycin*
escitalopram	esomeprazole	estazolam	estradiol
estradiol valerate	estrogens	eszopiclone	ethinyl estradiol
ethosuximide	etonogestrel	etoposide	etravirine
everolimus	exemestane	felbamate	felodipine
fentanyl	fesoterodine	fexofenadine	finasteride
fingolimod	flunisolide	flurazepam	flutamide
fluticasone	~~fosamprenavir~~*	fosaprepitant	fulvestrant
galantamine	gefitinib	granisetron	guanfacine
haloperidol*	hydrocodone	hydrocortisone	ifosfamide
iloperidone	imatinib*	imipramine	indacaterol
~~indinavir~~*	irinotecan	isosorbide dinitrate	isosorbide mononitrate
isradipine	itraconazole*	ivacaftor	ixabepilone
ketamine	ketoconazole*	lansoprazole	lapatinib

CYP3A4 INTERACTION TABLE (Ascorbate may decrease the plasma/blood concentrations of substrates) Drugs with ~~strikeout~~ text are antiretroviral drugs that are not allowed for use on this study. * 3A4 substrate & inhibitor; # 3A4 substrate & inducer			
lercanidipine	letrozole	levonorgestrel	lidocaine*
linagliptin	lomitapide	loperamide	~~lopinavir~~
loratadine	losartan	lovastatin	lumefantrine
lurasidone	~~maraviroc~~	marijuana	medroxyprogesterone
mefloquine	meloxicam	mestranol	methadone
methylergonovine	methylprednisolone	miconazole*	midazolam
mifepristone	mirabegron	mirtazapine	modafinil
mometasone	montelukast	nateglinide	nefazodone*
~~nelfinavir~~*	~~nevirapine~~#	nicardipine*	nifedipine
nilotinib	nimodipine	nisoldipine	nitrendipine
norethindrone	norgestrel	nortriptyline	omeprazole
ondansetron	ospemifene	oxybutynin	oxycodone
paclitaxel	paliperidone	palonosetron	pantoprazole
paricalcitol	paroxetine	pazopanib	perampanel
perphenazine	pimozide	pioglitazone	pomalidomide
ponatinib	prasugrel	prednisolone	prednisone
primaquine	progesterone/ progestins	propafenone	propranolol
quazepam	quetiapine	quinidine*	quinine
rabeprazole	ramelteon	ranolazine	regorafenib
repaglinide	rifabutin#	~~rilpivirine~~	risperidone
~~ritonavir~~*	rivaroxaban	roflumilast	romidepsin
ruxolitinib	salmeterol	~~saquinavir~~*	saxagliptin
selegiline	sertraline	sibutramine	sildenafil
silodosin	simvastatin	sirolimus	sitagliptin
solifenacin	sorafenib	sufentanil	sunitinib
tacrolimus	tadalafil	tamoxifen	tamsulosin
~~telaprevir~~	telithromycin*	temsirolimus	teniposide
terfenadine	testosterone	tetracycline*	theophylline
tiagabine	ticagrelor	ticlopidine	tinidazole
~~tipranavir~~	tofacitinib	tolterodine	tolvaptan
topotecan	toremifene	tramadol	trazodone
triazolam	trimethoprim	trimetrexate	trimipramine
ulipristal	vandetanib	vardenafil	vemurafenib
venlafaxine	verapamil*	vilazodone	vinblastine
vincristine	vinorelbine	vismodegib	voriconazole
warfarin	zaleplon	zileuton	ziprasidone
zolpidem	zonisamide	zopiclone	

PROTOCOL APPENDIX C: Data and Safety Monitoring Plan

Type of Clinical Trial:

- ☑ Investigator-initiated (UI/HCCC)
- ❑ Investigator-initiated, participating site
- ❑ Pilot study
- ❑ Phase I
- ❑ Phase I/II
- ☑ Phase II
- ❑ Phase III
- ❑ Compassionate-use/Expanded Access
- ☑ Interventional Treatment
- ❑ Interventional Non-Treatment
- ❑ Non-Interventional

Study risk-level:

- ❑ Level 1—low risk of morbidity or death, * <1% of death or any adverse event
- ❑ Level 2—risk of death* <1% or any adverse event 1% – 5%
- ❑ Level 3—risk of death* 1% – 5% or grade 4 – 5 SAE 1% – 5%
- ☑ Level 4—risk of death* >5% or grade 4 – 5 SAE >5%
- ❑ Drugs being used on a "compassionate" basis

***** *Risk of death" refers specifically to 100-day treatment-related mortality*

Reporting and Monitoring Requirements:

All institutional investigator initiated trials (IITs), regardless of assigned risk level are subject to routine DSMC monitoring activities which may include but are not limited to review of signed consent documents, eligibility and adverse event reporting.

All institutional IITs have the following **reporting requirements** as part of their DSMP:

- Provide an annual progress report to the DSMC and PRMC
- Register subjects in HCCC's Clinical Trial Management System, OnCore
- Document Adverse Events
- Document protocol deviations

Selected monitoring strategy based on risk-level:

Risk Level 4

Interventional treatment trials involving investigational agents or devices with a risk of death* (>5% or grade 4 – 5 SAE >5%), e.g. all investigator initiated INDs, most Phase I/II trials, gene therapy, gene manipulation or viral vector systems high-risk clinical procedures if performed solely for research purposes. The use of a new chemical or drug for which there is limited or no available safety data in humans.

Study Safety Review

An independent study monitor and/or the DSMC Chair (or designee), will review study data (provided by the PI/available in OnCore) and communicate with the PI at least biannually. A copy of this communication will be forwarded to the DSMC and PRMC Chairs.

Additional Reporting Requirements:

- A scanned copy of the completed eligibility checklist, with screening information and PI signature, will be attached in OnCore for ongoing review by DSMC staff.
- Serious adverse events will be entered directly into an OnCore SAE report by the research team. OnCore will send an automatic notification to the DSMC Chair/acting Chair and staff for review.
- The DSMC utilizes a risk-based monitoring approach. The trial's research records will be monitored at minimum twice per year. Monitoring may be done more frequently depending on the protocol, risks to subjects, reported serious/adverse events, patient population and accrual rate. Records for a minimum of 25% of subjects will be monitored for the entire study.

  Monitoring will involve the following:

  - review eligibility of patients accrued to the study,
  - check for the presence of a signed informed consent,
  - determine compliance with protocol's study plan,
  - determine whether SAEs are being appropriately reported to internal and external regulatory agencies,
  - compare accuracy of data in the research record with the primary source documents,
  - review investigational drug processing and documentation,
  - assess cumulative AE/SAE reports for trends and compare to study stopping rules.

Routine Adverse Event Reporting

For non-serious Adverse Events, documentation must begin from the first day of study treatment and typically continue through the 30 day follow-up period after the last dose of study drug (ascorbate).

Collected information should be recorded in the electronic/Case Report Forms (eCRF/CRF) for that subject. A description of the event, its severity or toxicity grade (according to NCI's Common Toxicity Criteria (CTCAE)), onset and resolved dates (if applicable), and the relationship to the study drug should be included. Documentation should occur in real time. The principal investigator has final responsibility for determining the attribution of the event as it is related to the study drug.

Serious Adverse Event Reporting

For any experience or condition that meets the definition of a serious adverse event (SAE), recording of the event must begin after signing of the informed consent and continue through the 30 day follow-up period after treatment is discontinued.

Investigators must report to the DSMC any serious adverse events (SAE), whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64). SAEs must be reported via an OnCore SAE Report within 24 hours of learning of the event.

An adverse event is considered **serious** if it results in ANY of the following outcomes:

1. Death
2. A life-threatening adverse event
3. An adverse event that results in inpatient hospitalization OR prolongation of existing hospitalization for ≥ 24 hours
4. A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5. A congenital anomaly/birth defect.
6. Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

FDA Reporting Requirements (for Sponsor-Investigators)

It is the responsibility of the IND sponsor-investigator to comply with IND safety reporting as set forth in the Code of Federal Regulations, Section 312.32. This responsibility includes providing an annual IND report to the FDA.

All IND safety reports must be submitted on Form 3500A and be accompanied by Form 1571. The type of report (initial or follow-up) should be checked in the respective boxes on Forms 3500A and 1571. See Instructions for completing Form 3500A. Please note all instance of UIHC, location, and faculty / staff should be redacted from supporting documentation and the 3500A.

The submission must be identified as:

- "IND safety report" for 15-day reports, or
- "7-day IND safety report" for unexpected fatal or life-threatening suspected adverse reaction reports, or
- "Follow-up IND safety report" for follow-up information.

For detailed explanation of the above definitions, requirements, and procedures related to IND application safety reports and the responsibilities of IND applications sponsors with regard to such reporting, refer to Guidance for Industry and Investigators: Safety Reporting Requirements for INDs and BA/BE Studies (PDF - 227KB)

In addition to completing appropriate patient demographic and suspect medication information, the report should include the following information within the Event Description (section 5) of the MedWatch 3500A form:

- Treatment regimen (dosing frequency, combination therapy)
- Protocol description (and number, if assigned)
- Description of event, severity, treatment, and outcome, if known (grading the event per CTCAE)
- Supportive laboratory results and diagnostics
- Sponsor-Investigator's assessment of the relationship of the adverse event to each investigational product and suspect medication

Data Monitoring and Management

All studies that undergo PRMC review and/or utilize HCCC Clinical Research Services (CRS) resources are required to register subjects in OnCore. Subject registration includes the following:

- Consent date and the IRB approved consent used
- Date of eligibility and eligibility status (eligible, not eligible)
- On study date and subject's disease site (and histology if applicable)
- On treatment date (if applicable)

Subject Data

In addition to the subject registration and subject status data entered in OnCore for all HCCC trials, research staff also enters the subject study data into electronic case report forms (eCRFs) for HCCC investigator initiated studies. eCRFs are approved by the PI and statistician prior to study activation to ensure the most effective data acquisition. All information on eCRFs will be traceable to the source documents which are generally maintained in the subject's file. eCRF data are expected to be entered into OnCore within 30 calendar days after a subject's study visit.

Forms Monitoring

OnCore eCRF data are monitored on a routine basis (dependent on accrual) to ensure all mandatory fields are entered completely, accurately and within time requirements. The assigned DSMC monitor manages the logistics associated with the data monitoring review. Once the clinical trial is identified for monitoring, the monitor arranges for a selection of cases to review from among the subjects registered in OnCore. As part of the forms monitoring process, the assigned monitor will issue queries within the eCRF to resolve missing, incomplete and/or incorrect information. A member of the research team is expected to respond to monitoring queries within 14 business days.

This process can often identify a misunderstanding or deficiency in protocol requirements early in the study and can improve data quality.

Final Reports

A summary of each subject's data record is continually available to the PI, research staff, and DSMC from OnCore's Biostat Console. The availability of this information is a valuable tool for the preparation of final reports and manuscripts as well as ongoing deficiency reports.

References:

1. Siegel RL, Miller KD, Jemal A: Cancer statistics, 2016. CA Cancer J Clin 66:7-30, 2016
2. Riedel RF: Systemic therapy for advanced soft tissue sarcomas: highlighting novel therapies and treatment approaches. Cancer 118:1474-85, 2012
3. McCormick W: Cancer: the preconditioning factor in pathogenesis. Arch Pediat 71:313-322, 1954
4. McCormick W: Cancer: a collagen disease, secondary to a nutritional deficiency? . Arch Pediat 76:166-171, 1959
5. Cameron E, Rotman, D: Ascorbic acid, cell proliferation, and cancer. Lancet 299:542, 1972
6. Cameron E, Pauling, L: Ascorbic acid and the glycosaminoglycans. An orthomolecular approach to cancer and other diseases. Oncology 27:181-192, 1973
7. Cameron E, Pauling, L: Supplemental ascorbate in the supportive treatment of cancer: Prolongation of survival times in terminal human cancer. Proc Natl Acad Sci USA 73:3685-3689, 1976
8. Cameron E, Campbell, A: The orthomolecular treatment of cancer. II Clinical trial of high-does ascorbic acid supplements in advanced human cancer. Chem Biol Interact 9:285-315, 1974
9. Cameron E, Pauling L: Supplemental ascorbate in the supportive treatment of cancer: reevaluation of prolongation of survival times in terminal human cancer. Proceedings of the National Academy of Sciences of the United States of America 75:4538-42, 1978
10. Cameron E, Campbell, A:: Innovation vs. quality control: an 'unpublishable' clinical trial of supplemental ascorbate in incurable cancer Med Hypotheses 36:185-189, 1991
11. Campbell A, Jack, T, Cameron, E: Reticulum cell sarcoma: two complete 'spontaneous' regressions, in response to high dose ascorbic acid therapy. A report on subsequent progress. Oncology 48:495-497, 1991
12. Murata A, Morishige, F, Yamaguchi, H: Prolongation of survival times of terminal cancer patient by administration of large doses of ascorbate. Int J. Vitam Nutr Res Suppl 23:103-113, 1982
13. Creagan E, Moertel, CG, O'Fallon, JR, Schutt, AJ, O'Connell, MJ, Rubin, J, Frytak, S: Failure of high-dose vitamin C (ascorbic acid) therapy to benefit patients with advanced cancer: A controlled trial. N Engl J Med 301:687-690, 1979
14. Moertel C, Flemin, TR, Creagan, ET, Rubin, J, O'Connell, MJ, Ames, MM: : High-dose vitamin C versus placebo in the treatment of patients with advanced cancer who have had no prior chemotherapy. A randomized double-blind comparison. N Engl J Med 312:137-141, 1985
15. Levine M, Conry-Cantilena, C, Wang, Y, Welch, RW, Washko, PW, Dhariwal, KR, Park, JB, Lazarev, A, Graumlich, JF, King, J, Cantilena, LR. : Vitamin C Pharmacokinetics in healthy volunteers: Evidence for a recommended dietary allowance. Proc Natl Acad Sci USA 93:3704-3709, 1996
16. Padayatty SJ, Levine, M: Reevaluation of vitamin C in cancer treatment: emerging evidence, open minds and serendipity. J. Amer. Coll. Nutr. 19:423-425, 2000
17. Graumlich J, Ludden, TM, Conry-Cantilena, C, Cantilena, Jr LR, Wang, Y, Levine, M: Phamacokinetic model fo ascorbic acid in healthy male volunteers during depletion and repletion. Pharm Res 14:1133-1139, 1997
18. Riordan N, Riordan, HD, Meng, X, Li, Y, Jackson, JA: Intravenous ascorbate as a

tumor cytotoxic chemotherapeutic agent. Med Hypotheses 44:207-213, 1995

19. Chen Q, Espey, MG, Krishna, MC, Mitchel,l JB, Corpe, CP, Buettner, GR, Shacter, E, Levine, M. : Pharmacologic ascorbic acid concentrations selectively kill cancer cells: Action as a pro-drug to deliver hydrogen peroxide to tissues. Proc Natl Acad Sci USA 102:13604-13609, 2005

20. Chen Q, Espey, MG, Sun, AY, Lee, JH, Krishna, MC, Shacter, E, Choyke, PL, Pooput, C, Kirk, KL, Buettner, GR, Levin, M.: Ascorbic acid in pharmacologic concentrations: a pro-drug for selective delivery of ascorbate radical and hydrogen peroxide to extracellular fluid in vivo Proc Natl Acad Sci USA 104:8749-8754, 2007

21. Padayatty S, Sun, H, Wang, Y, Riordan, HD, Hewitt, SM, Katz, A, Wesley, RA, Levine, M. : Vitamin C pharmacokinetics: implications for oral and intravenous use. Annals of Internal Medicine 140:533-537, 2004

22. Chen Q, Sun, AY, Espey, MG, Levine, M: Ascorbic acid as a pro-oxidant therapeutic agent in cancer. Free Radical Biology and Medicine 43:S52, 2007

23. Hoffer LJ, Levine M, Assouline S, et al: Phase I clinical trial of i.v. ascorbic acid in advanced malignancy. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO 19:1969-74, 2008

24. Welsh JL, Wagner BA, van't Erve TJ, et al: Pharmacological ascorbate with gemcitabine for the control of metastatic and node-positive pancreatic cancer (PACMAN): results from a phase I clinical trial. Cancer Chemother Pharmacol 71:765-75, 2013

25. Heinemann V, Hertel LW, Grindey GB, et al: Comparison of the cellular pharmacokinetics and toxicity of 2',2'-difluorodeoxycytidine and 1-beta-D-arabinofuranosylcytosine. Cancer Res 48:4024-31, 1988

26. Iwasaki H, Huang P, Keating MJ, et al: Differential incorporation of ara-C, gemcitabine, and fludarabine into replicating and repairing DNA in proliferating human leukemia cells. Blood 90:270-8, 1997

27. Gandhi V, Legha J, Chen F, et al: Excision of 2',2'-difluorodeoxycytidine (gemcitabine) monophosphate residues from DNA. Cancer Res 56:4453-9, 1996

28. Abbruzzese JL, Grunewald R, Weeks EA, et al: A phase I clinical, plasma, and cellular pharmacology study of gemcitabine. J Clin Oncol 9:491-8, 1991

29. Tempero M, Plunkett W, Ruiz Van Haperen V, et al: Randomized phase II comparison of dose-intense gemcitabine: thirty-minute infusion and fixed dose rate infusion in patients with pancreatic adenocarcinoma. J Clin Oncol 21:3402-8, 2003

30. Patel SR, Gandhi V, Jenkins J, et al: Phase II clinical investigation of gemcitabine in advanced soft tissue sarcomas and window evaluation of dose rate on gemcitabine triphosphate accumulation. J Clin Oncol 19:3483-9, 2001

31. Merimsky O, Meller I, Kollender Y, et al: Palliative effect of gemcitabine in osteosarcoma resistant to standard chemotherapy. Eur J Cancer 34:1296-7, 1998

32. Amodio A, Carpano S, Manfredi C, et al: [Gemcitabine in advanced stage soft tissue sarcoma: a phase II study]. Clin Ter 150:17-20, 1999

33. Wagner-Bohn A, Paulussen M, Vieira Pinheiro JP, et al: Phase II study of gemcitabine in children with solid tumors of mesenchymal and embryonic origin. Anticancer Drugs 17:859-64, 2006

34. Weitman S, Ochoa S, Sullivan J, et al: Pediatric phase II cancer chemotherapy trials: a Pediatric Oncology Group study. J Pediatr Hematol Oncol 19:187-91, 1997

35. Maki RG, Wathen JK, Patel SR, et al: Randomized phase II study of gemcitabine

and docetaxel compared with gemcitabine alone in patients with metastatic soft tissue sarcomas: results of sarcoma alliance for research through collaboration study 002 [corrected]. J Clin Oncol 25:2755-63, 2007

36. Bauer S, Seeber S, Schutte J: Gemcitabine in the treatment of soft tissue sarcomas. Onkologie 27:180-6, 2004

37. van Heeswijk RP, Cooper CL, Foster BC, et al: Effect of high-dose vitamin C on hepatic cytochrome P450 3A4 activity. Pharmacotherapy 25:1725-8, 2005

38. Eisenhauer EA, Therasse P, Bogaerts J, et al: New response evaluation criteria in solid tumours: revised RECIST guideline (version 1.1). European Journal of Cancer 45:228-47, 2009