Phase 1b/2 Neoadjuvant High Dose Ascorbate with Concurrent Preoperative Radiation in Patients with Locally Advanced Soft Tissue Sarcomas of Extremity, Trunk and Retroperitoneum

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Study Agent: Pharmacological ascorbate

Other Agent(s): Standard external beam radiation

Investigational Agent	IND#	IND Sponsor
Ascorbic acid	137968	Mohammed Milhem

Protocol Version 8

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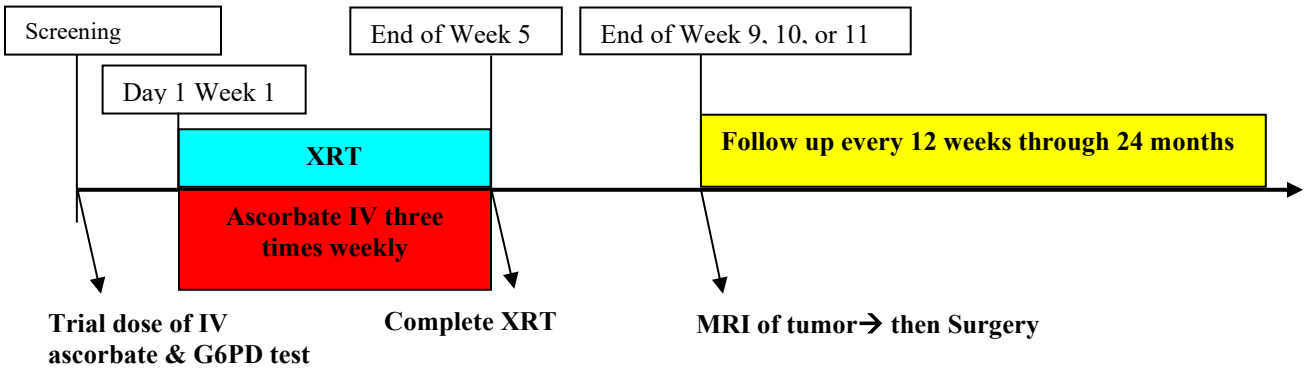
SCHEMA

Study Timeline Schema:



Phase 1b Schema:

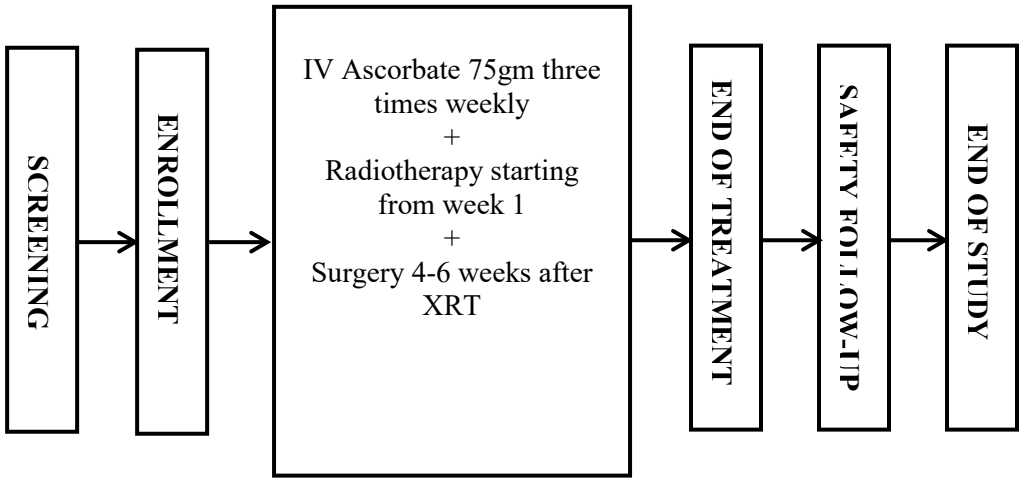


Table 1. Phase 1b Dose De-escalation Schedule

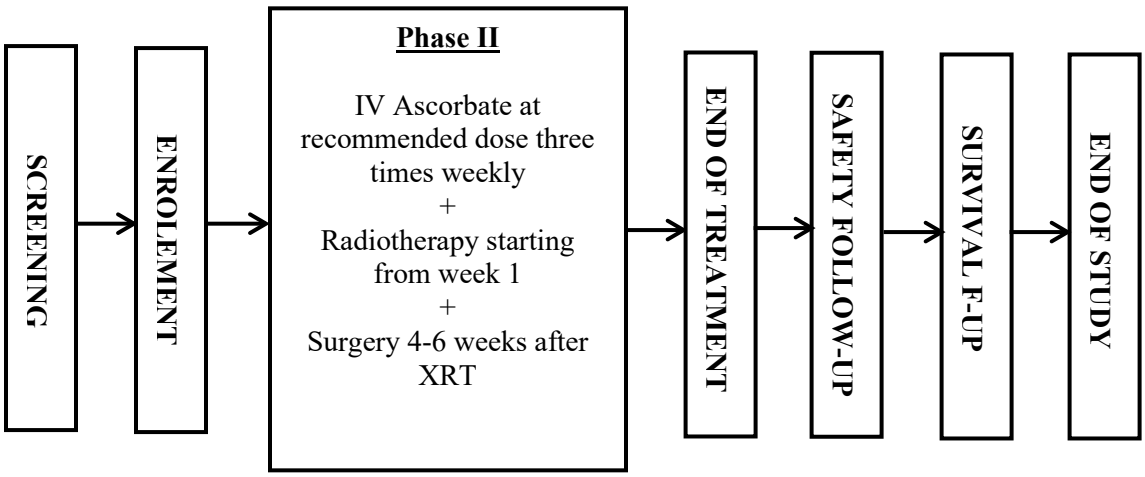
Number of patients with DLT at a given dose level	Decision Rule
0 out of 3	Enter 3 patients at the next higher dose level
1 out of 3	Enter up to 3 additional patients at the same dose level. • If 0 of these 3 patients experience DLT, proceed to the next dose level. • If 1 or more of these 3 patients experience DLT, then dose escalation is stopped and this dose is declared the maximally administered dose. Three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
≥2 out of 3	Dose escalation will be stopped. This dose level will be declared the maximally administered dose. Three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.

Ascorbate Dose Levels:

Dose 0 = Ascorbate 75 gm IV three times weekly concurrently with radiation
Dose -1 = Ascorbate 62.5 gm IV three times weekly concurrently with radiation

- Initial dose for all = Ascorbate 75gm IV

Phase 2 Schema:



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1. BACKGROUND

1.1 Disease Background

Adult soft tissue sarcomas (STS) constitute a wide heterogeneous group of very rare tumors, both in terms of histology and biological and clinical behavior. 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. 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 one year and only about 10% of these patients are alive at five years[1].

Control of localized disease is, therefore, of prime importance as evidenced by the National Comprehensive Cancer Network's (NCCN) current treatment guidelines[2]. Pisters et al demonstrated that histologic grade, depth of location, and positive surgical margins are key determinants of STS recurrence.[3] However, patients typically present late with local tumor extensions into critical adjacent structures, making complete surgical resection with uninvolved margins difficult. Preoperative radiotherapy has been shown to significantly improve local disease control, functional outcomes and survival in patients with locally advanced STS.[4-6]

1.2 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,[7,8] while Cameron hypothesized that ascorbate could have anti-cancer action by inhibiting hyaluronidase and thereby prevent cancer spread.[9] These hypotheses were subsequently promoted by Cameron and Pauling.[10,11] Cameron and Campbell initially published case reports of 50 patients; some seemed to have benefited from high dose ascorbate.[12] Cameron and Pauling then published results of 100 patients with terminal cancer that were given intravenous ascorbate.[13] 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.[11,13] 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.[14] Smaller studies have also reported benefits of ascorbate.[15,16]

To test "definitively" whether ascorbate was effective, Moertel conducted two randomized placebo-controlled studies randomized to <u>oral</u> ascorbate; neither study showed benefit.[17,18] 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.[19,20] This difference in the administration route is key. Cameron gave patients ascorbate <u>intravenously</u> as well as orally, while Moertel's patients received only <u>oral</u> ascorbate. Thus, the issue of ascorbate in cancer treatment needed 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.[21] As doses exceed 200 mg, absorption decreases, urine excretion increases and ascorbate bioavailability is reduced.[19,21] 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.[22] 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.[23] 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.[24] 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.[25] *In vivo*, Chen and colleagues demonstrated that intravenous injection of ascorbate (0.25-0.5 mg/g body weight) increased baseline concentrations of ascorbate in the blood and extracellular fluid to > 8 mM as well as increased formation of H_2O_2.[26] 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.[21] Our data demonstrate that pharmacologic doses of ascorbate are cytotoxic to sarcoma cell lines and that it enhances radiation therapy sensitivity *in vitro* **(Figure 1).** Additionally, we also showed that intraperitoneal administration of high dose ascorbate in combination with radiation inhibits orthotopic sarcoma growth and decreases tumor size in mice without causing an associated toxicity *in vivo* **(Figure 2).** Combined, these studies provide a foundation for pursuing pharmacologic ascorbate as a prooxidant agent in cancer therapy and specifically in sarcoma.[27]

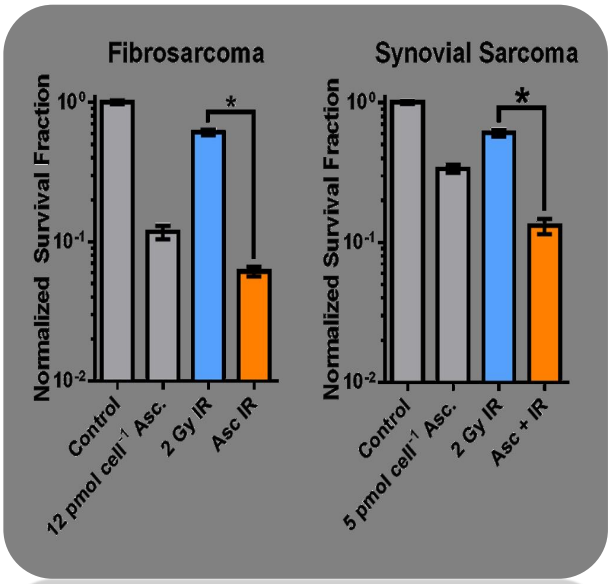


Figure 1 Demonstrates that sarcoma cell lines, fibrosarcoma (HT1080) and synovial sarcoma (SW-872), are sensitive to increasing doses of pharmacological ascorbate. Cells were treated with increasing doses of ascorbate for 1 hour followed by clonogenic cell survival analysis.

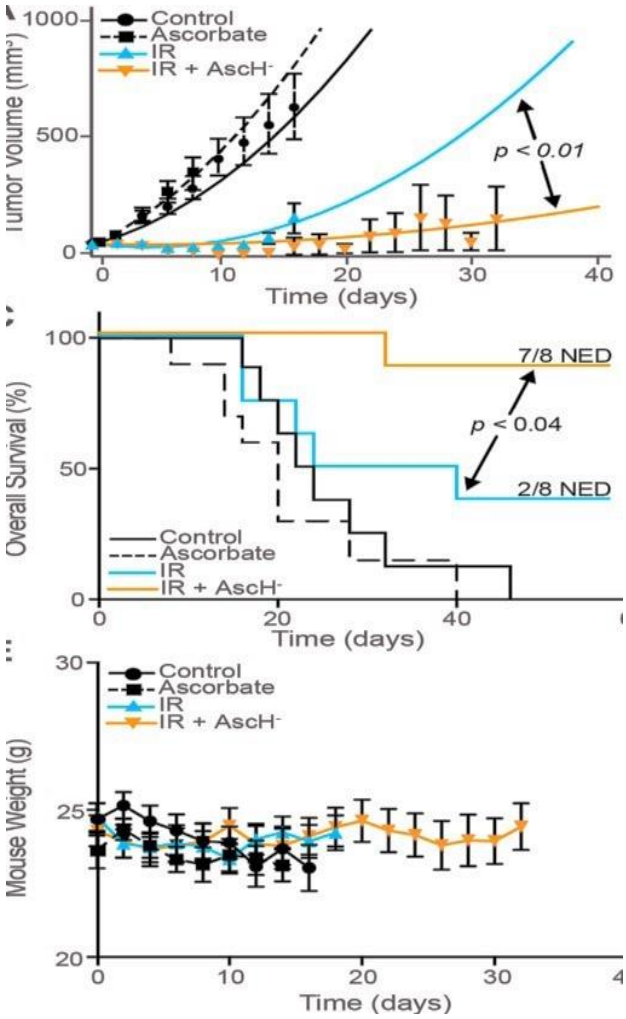


Figure 2 Pharmacological ascorbate selectively sensitizes fibrosarcoma tumors to radiation in an orthotopic murine xenograft model. An orthotopic fibrosarcoma model was utilized by injecting 1×106 HT-1080 cells into the dermis of the right rear flank of athymic nude female mice. Once tumors were established, therapy was initiated with daily ascorbate (4 g kg−1 or sodium chloride control; IP) in combination with 12 Gy IR in 2 fractions (days 2 and 4) (A) Tumor volume measurements (points +/- error) fitted with a logistical regression (line) for mice treated with daily ascorbate in combination with radiation (B) Overall survival in mice treated with ascorbate in combination with radiation. Mice were sacrificed when any tumor dimension reached 1.5 cm. NED=no evidence of macroscopic disease at end of experiment. (C) Mouse weight in mice treated with ascorbate in combination with radiation. For each treatment group, n = 8 mice.

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.[23,24,26] When ascorbate is infused intravenously the resulting pharmacologic concentration distributes rapidly in the extracellular water space.[26] 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.[18] 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.

1.3 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.[28] 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.[29]

A phase I study of adding ascorbate to standard therapy in glioblastomas and NSCLC was initiated in 2013.[30] Subjects were assigned to high-dose ascorbate dose cohorts for the concomitant portion of therapy. Dose cohorts ranged from 15g to 87.5g. During the adjuvant phase, subjects underwent interpatient dose escalation to achieve a targeted plasma ascorbate level of 350 mg/dL. Subjects received high-dose ascorbate during the adjuvant phase for up to 6 cycles. To date, 5 subjects have been enrolled in the recommended phase 2 dose of 87.5g. Treatment was well-tolerated with minimal adverse events. No subject experienced a grade 3 or greater hematologic or non-hematologic toxicity. The only adverse events associated with ascorbate were grade 1 dry mouth, grade 1 chills, and grade 2 hypertension. Dry mouth is transient and ends shortly after infusion. Grade 1 chills are infusion related, most likely due to the volume of fluid infused. Grade 2 hypertension occurs in some subjects during infusion, again most likely due to the volume of fluid infused.[30]

1.4 Radiation Therapy in Sarcoma

The benefits of combination therapy with radiation and surgery in significantly improving local control in STS patients are well documented. Preoperative radiotherapy has been shown to significantly improve local disease control, functional outcomes and survival in patients with locally advanced STS[4-6]. Recent review of literature indicates that about 10% of patients will have ≥ 95% tumor necrosis, and about 25% will achieve ≥ 80% tumor necrosis following preoperative radiation therapy alone.[31,32] Further review indicates that only those patients who achieve ≥ 95% tumor necrosis with preoperative radiation have improved local and distant control as well as overall survival.[32] Despite its reported benefits, radiotherapy is associated with a high rate of complications and significant functional morbidity. O'Sullivan et al compared preoperative versus postoperative external beam radiation (EBRT) for resectable extremity STS. Long term follow up revealed no statistical difference in local control and cause specific survival, however, preoperative radiotherapy was associated with a significantly higher rate of acute wound complications (35% vs. 17%, $p = 0.01$) and a lower rate of late Grade 2– 4 fibrosis (32% vs. 48%, $p = 0.07$).[33,34] Multiple retrospective reviews have similarly found high rates of acute wound complications in sarcoma patients ranging from 25% to 44%.[35-38] These reported high rates of acute and late toxicities support the need for additional research to improve the safety profile of neoadjuvant radiotherapy.

In addition, there is need to improve efficacy of the radiation treatments to achieve higher rates of tumor necrosis, and, to ensure resection of the tumor with clear margins for successful organ preserving treatment of sarcoma.

2. REGISTRATION PROCEDURES

NA; Single Institution study.

3. OBJECTIVES

OBJECTIVES	ENDPOINTS
Primary	
Phase 1b: To determine the safety and tolerability of neoadjuvant ascorbate in combination with preoperative EBRT as assessed by incidence of dose-limiting toxicities (DLT) in subjects with locally advanced high grade soft tissue sarcomas of extremity, trunk and retroperitoneum.	Incidence of Dose Limiting Toxicities.
Phase 2: To estimate the efficacy of neoadjuvant ascorbate and radiotherapy as assessed by the pathological complete response rates (pCR) in subjects with locally advanced high grade soft tissue sarcomas. For this study, pCR will be defined as ≥ 95% tumor necrosis following concurrent radiation therapy and ascorbate.	pCR rate- the proportion of subjects with pathologic tumor necrosis ≥ 95%.
Secondary	
Time to disease progression (local or distant recurrence)	Time from first day of study treatment to first documented disease progression.
Overall response rate (ORR) pre op as measured by RECIST 1.1 or a later tool for monitoring disease progression.	The proportion of subjects with a complete or partial response.
Overall survival rate (OS) at 2 Years	Time from first day of study treatment to death due to any cause. Survival will be estimated at 2 years.
To grade radiation related skin toxicity overlying the tumor	Incidence of radiation related skin toxicity by grade

OBJECTIVES	ENDPOINTS
To measure labile iron using T2* imaging sequence on MRI pre and post ascorbate treatments and compare with serum iron measurements	Changes in labile iron and serum iron from pre to post-treatments
To evaluate diffusion weighted imaging sequences on MRI in pre and post treatment tumors	Changes in diffusion weighted imaging sequence from pre-to post-treatment

3.1 Primary Objectives

Phase 1b: To determine the safety and tolerability of neoadjuvant ascorbate in combination with preoperative EBRT as assessed by incidence of dose-limiting toxicities (DLT) in subjects with locally advanced high grade soft tissue sarcomas of extremity, trunk and retroperitoneum.

Phase 2: To estimate the efficacy of neoadjuvant ascorbate and radiotherapy as assessed by the pathological complete response rates (pCR) in subjects with locally advanced high grade soft tissue sarcomas. For this study, pCR will be defined as ≥ 95% tumor necrosis following concurrent radiation therapy and ascorbate.

3.2 Secondary Objectives

- Time to disease progression (local or distant recurrence)
- Overall response rate (ORR) pre op as measured by RECIST 1.1 or a later tool for monitoring disease progression.
- Overall survival rate (OS) at 2 years
- To grade radiation related skin toxicity overlying the tumor as compared to historical controls
- To measure labile iron using T2* imaging sequence on MRI pre and post ascorbate treatments and compare with serum iron measurements
- To evaluate diffusion weighted imaging sequences on MRI in pre and post treatment tumors and correlate it with necrosis and survival

4. PATIENT SELECTION

4.1 Inclusion Criteria

4.1.5 Subject or subject's legally acceptable representative has provided informed consent.

4.1.6 Histologically confirmed diagnosis of locally advanced soft tissue sarcoma of extremity, trunk or retroperitoneum that is unresectable with clear wide margins, for which preoperative radiotherapy is considered appropriate.

- Including metastatic (stage IV) disease for which radiotherapy and surgical resection of the primary tumor are indicated.

4.1.7 Patients with locally recurrent sarcoma after surgery alone are eligible for enrollment if

other inclusion criteria are met.

4.1.8 Patient does not have histologic subtypes: GIST, Desmoid, Ewing sarcoma, bone sarcomas and Kaposi sarcoma

4.1.9 Age ≥18 years.

4.1.10 Patients with history of non-melanomatous skin cancer, in situ carcinoma, or low-risk prostate cancer can be enrolled.

4.1.11 ECOG performance status ≤1.

4.1.12 Tolerate one test dose (15g) of ascorbate.

4.1.13 Patient must have measurable disease:

- Tumor size at least ≥ 5 cm in the longest diameter as measured by CT scan or MRI for which radiation is feasible and indicated.

4.2 Exclusion Criteria

4.2.1 Inadequate organ function within 21 days of Day 1 of study as defined by:

- Hemoglobin < 9.0 g/dL
- Absolute neutrophil count (ANC) </= 1500 per mm^3
- Platelet count </= 100,000 per mm^3
- Total bilirubin >/= 1.5 × ULN. Subjects with direct bilirubin < ULN with total bilirubin levels > 1.5 X ULN will not be excluded.
- Aspartate aminotransferase (AST) or alanine aminotransferase (ALT) > 2.5 × ULN
- Alkaline phosphatase > 2.5 × ULN
- PT (or INR) and PTT (or aPTT) >/= 1.5 × ULN
- Creatinine > 2.0 × ULN

4.2.2 G6PD (glucose-6-phosphate dehydrogenase) deficiency

4.2.3 Prior history of two episodes of symptomatic oxalate kidney stones within last year.

4.2.4 Prior radiation therapy in excess of 20 Gy to the site of the current diagnosis of sarcoma. No overlap with prior radiation fields in excess of 20 Gy is allowed.

4.2.5 Prior history of receiving pharmacological ascorbate.

4.2.6 Patients actively receiving insulin therapy AND needing daily fingerstick for glucose monitoring.

4.2.7 Concurrent, clinically significant, active malignancies within two years of study enrollment.

4.2.8 Female subjects who are pregnant or breast-feeding, or planning to become pregnant during study treatment and through 3 months after the last dose of study treatment.

4.2.9 Female subjects of childbearing potential or male subjects who are unwilling to use 2 highly effective methods of contraception during study treatment and through 3 months after the last dose of study treatment.

4.2.10 Currently receiving treatment in another invasive investigational device or drug study, or less than 30 days since ending treatment on another investigational device or drug study(s).

4.2.11 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.

4.2.12 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

4.2.13 History of allergic reactions attributed to compounds of similar chemical or biologic composition to ascorbate

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

4.2.15 Known HIV-positive and hepatitis B & C individuals. High-dose ascorbate acid is a known CYP450 3A4 inducer, which results in lower serum levels of antiretroviral drugs

4.2.16 Patients who are on warfarin and cannot have a drug substitution or who decline the drug substitution.

5. TREATMENT PLAN AND STUDY SCHEMA

5.1 Study Design

This is a single-arm open-label phase Ib/II clinical study assessing the efficacy of concurrent high dose ascorbate in combination with radiotherapy in patients with locally advanced, resectable, high grade sarcomas, as measured by pathological response rates. Patients will be treated with three times weekly IV ascorbate concurrently with radiation therapy. Surgery will be performed 4-6 weeks from the end of radiation therapy to allow for resolution of acute toxicities per current standard of care.

5.2 Phase Ib

The phase Ib portion of this study is to ensure the safety and tolerability of high dose ascorbate in combination with EBRT as assessed by incidence of dose-limiting toxicities (DLT). Although this combination has been shown to be safe and well tolerated in GBM, this combination has not been studied in sarcoma patients. EBRT will be given at the standard dose for resectable soft tissue sarcomas according to the NCCN sarcoma guidelines.[2] Patients will receive 50 Gy over 25 fractions for extremity and trunk sarcomas or 45 – 50.4 Gy in 28 fractions for retroperitoneal sarcomas, during which time they will be receiving three times a week IV high dose ascorbate. IV ascorbate infusions will be continued until the end of radiation therapy. Surgery will be performed 4-6 weeks from the end of radiation to allow for adequate tissue healing and resolution of acute toxicities.

Ascorbate will be dosed at an initial dose of 75gm IV three times a week concurrently with radiation therapy provided patient tolerated the test dose of ascorbate during screening without any reactions. Initially 3 patients will be entered at the Dose 0 for ascorbate and be monitored at this three times a week dose until surgery when safety issues will be addressed. Otherwise, ascorbate dose will be deescalated and dose -1 will be explored as outlined. For this protocol DLT will be defined as an attributed adverse event (definite, probable or possible) that meets the criteria (Section 6.3).

Table 1. Phase 1b Dose De-escalation Schedule

Number of Patients with DLT at a Given Ascorbate Dose	**Decision Rule**
≥ 2	Dose level will be declared toxic. If this is the lowest dose level, stop the study due to excessive toxicity; otherwise enter *three* additional patients at the next lowest dose level.
≤ 1 out of 3	Enter *three* more patients at this dose level.
≤ 1 out of 6	This is the recommended dose for the subsequent Phase2.

Ascorbate Dose Levels

Dose 0	Ascorbate 75 gm IV three times weekly concurrently with radiation
Dose -1	Ascorbate 62.5 gm IV three times weekly concurrently with radiation

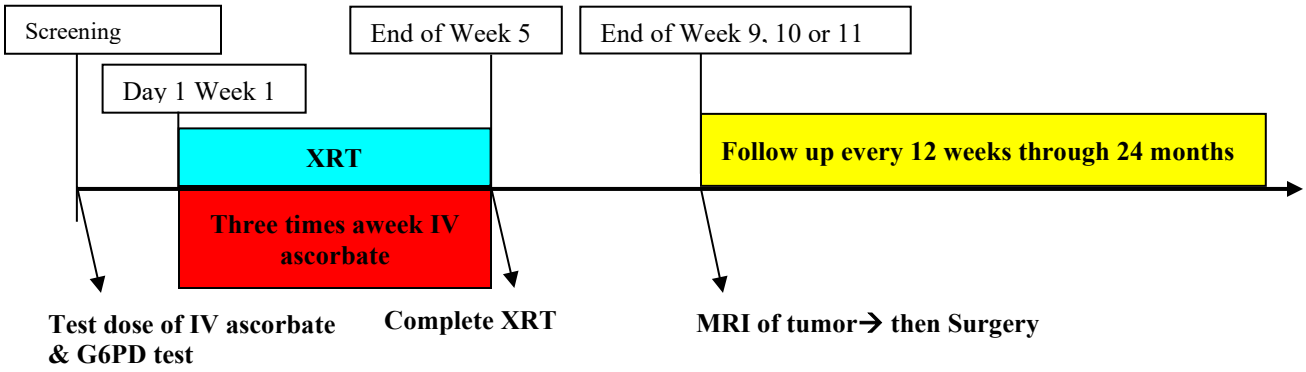
- Initial dose for all = Ascorbate 75gm IV

5.3 Phase 2

The phase 2 part of the study will provide an estimate of the relative treatment effect of pharmacological ascorbate in combination with preoperative EBRT in subjects with locally advanced, resectable, extremity or retroperitoneal high grade sarcomas, as measured by pathological response rates.

As above, patients will receive the first dose of pharmacological ascorbate intravenously on day 1 of week 1 provided no reactions are seen to the test dose. This will be followed by 3 times a week dosing (= 15 doses total) at Dose 0 until completion of EBRT. Standard doses of radiation for resectable soft tissue sarcomas according to the NCCN sarcoma guidelines will be administered.[2] Patients will receive preoperative radiation at a dose of 50 Gy over 25 fractions for extremity and trunk sarcoma or 45 to 50.4 Gy in 28 fractions for retroperitoneal sarcomas starting on week 1 day 1. Subjects should be followed either by clinic visit or phone contact every 12 weeks (+/- 4 weeks) for approximately 24 months after the end of the treatment phase, at which time the initial survival data and disease recurrence will be assessed. Details as per study calendar (Section 10).

5.4 Study Timeline Schema:



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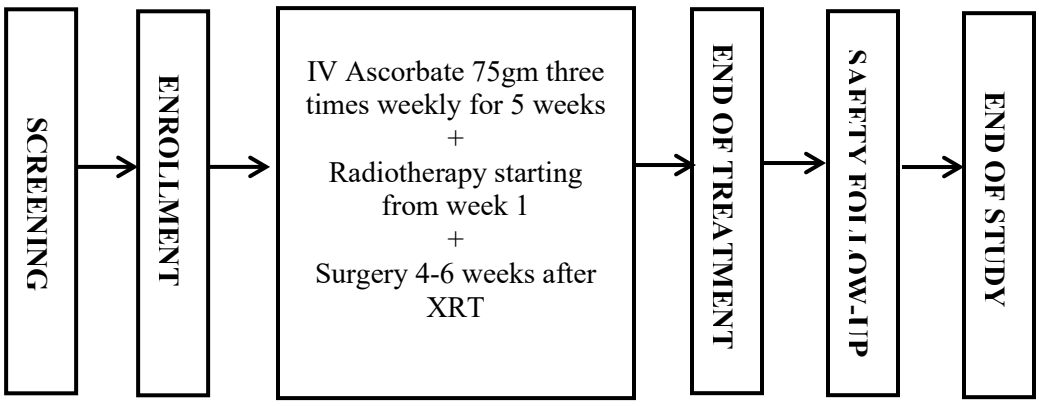
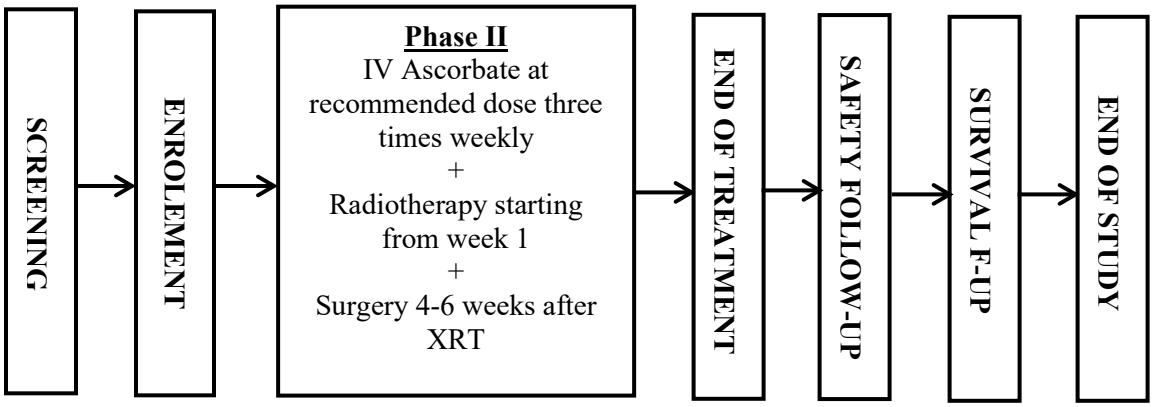


Phase 2 schema:



5.5 Post-consent Screening Procedures

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

- 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).
- One test dose of 15g of ascorbate. A physician must review the patient's medication list against the list of medications prohibited on this trial prior to the test dose of ascorbate.

5.6 Concomitant Ascorbate (High-dose Ascorbic Acid) Infusions

5.6.1 **Participant dose**. All patients will receive a standard ascorbate dose of 75 gm given over 120 minutes

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

5.6.3 **Initiation**. Start the week prior to chemoradiation with one 15-g infusion. This is the test dose. 75 gm dosing begins week 1 of radiation therapy. If the participant fails the 15g test dose it is not considered dose limiting toxicity.

5.6.4 **Schedule**. Scheduled three times weekly during chemoradiation;.

5.6.5 **Duration.** Subjects will continue with therapy until the end of standard radiation therapy or if any criteria of removal are met (Section 5.7)

5.6.6 **Dose modifications.** Provided in Section 6.2

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
62.5 g (125 mL)	1000 mL	709	120 mins

† 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

5.7 Radiation therapy

External beam radiation therapy will be given at the standard dose for resectable soft tissue sarcomas according to the NCCN sarcoma guidelines.[2] Patients with extremity and trunk STS will receive 50 Gy over 25 fractions and those with retroperitoneal STS will receive 45 to 50.4 Gy in 28 fractions, during which time they will be receiving three times weekly IV ascorbate until the end of radiation therapy. Surgery will be performed 4-6 weeks from the end of radiation to allow for adequate tissue healing and resolution of acute toxicities.

Radiation therapy for this protocol is as per currently used standard pre-operative radiation therapy at the University of Iowa even for non-protocol patients. Selected guidelines from previously conducted Radiation Therapy Oncology Groups (RTOG) studies are briefly summarized for this protocol.

5.7.1 **Preoperative Radiation therapy (3D-CRT or IMRT**). Either 3D conformal radiotherapy or intensity modulated radiation therapy may be utilized for adequate tumor coverage and to meet the dose constraints for critical normal structures.

5.7.2 **Radiation treatment planning scans and immobilization**. CT scans with patient in a stable and comfortable position will be used for planning. MRI scans are optional and may be used for co-registration with CT for tumor localization as needed. VacLoc or other immobilization devices can be used for immobilization.

5.7.3 **Target volumes and treatment field (Extremity Sarcoma):**

Gross Target Volume (GTV): Outline of the tumor as seen on CT or CT/MRI fusion.

Clinical Target Volume (CTV): CTV = GTV plus 4 cm margins in the proximal and distal directions. However, the margin can be reduced if it extends beyond the compartment, the field can be shortened to include the end of a compartment. The radial margin from the lesion should be 1 cm. It can be reduced in areas confined by the fascial barrier or bone or skin surface as per standard of care and treating radiation oncologist's discretion.

Planning Target Volume (PTV): Include CTV and error of setup and organ motion. Typically PTV includes CTV plus 0.5 - 1 cm in all dimensions. Final PTV is usually GTV + 5 cm longitudinal and 2 cm radial, except where restricted by the compartment end, fascia, bone or skin surface as per standard of care and treating radiation oncologist's discretion.

Use of bolus on the skin surfaces is optional as needed. Where applicable biopsy scars should be bolused with appropriate thickness specific to the energy of the photon beam.

5.7.4 **Target volumes and treatment field (Retroperitoneal/Trunk Sarcoma):**

Gross Target Volume (GTV): Outline of the tumor as seen on CT or CT/MRI fusion.

Clinical Target Volume (CTV): 4D-CT imaging should be performed for tumors above the iliac brim to assess for possible tumor motion associated with respiration. The CTV is generated by expanding the different respiratory phases GTV (ITV) by 1.5 cm symmetrically. The margin can be reduced if it extends beyond the retroperitoneal compartment into bone, kidney, or liver. If tumor extends through inguinal canal, an additional 3cm can be added distally as per standard of care and treating radiation oncologist's discretion.

Planning Target Volume (PTV): Include CTV and error of setup and organ motion. PTV expansions are typically CTV plus 0.5 - 1 cm in all dimensions. Final PTV is typically ITV + 2 cm symmetrically as confined to the retroperitoneal compartment as per standard of care and treating radiation oncologist's discretion.

5.7.5 **Dose Specifications**:

A dose of 50 Gy in 25 daily fractions for extremity STS and 45 to 50.4 Gy in 28 fractions for retroperitoneal STS will be prescribed to the planning target volume (PTV) using linear accelerator and megavoltage photon beams with energies of 6MV or greater.

For retroperitoneal/intra-abdominal sarcomas, a dose-painted simultaneous integrated boost (SIB) to total dose of 57.5 Gy in 25 fractions is allowed in select cases. A postoperative EBRT boost is discouraged.

Prescribed dose should cover > 90% of the PTV. More than 95% of the PTV should receive > 95% of the prescribed dose. No more than 20% of the PTV will receive ≥ 110% prescription dose.

5.7.6 **Precautions and Dose Constraints for critical structures**:

Radiation dose to normal tissues should be kept within the accepted normal tissue tolerances. Every effort should be made to:

a) Avoid treating the full circumference of an extremity;

b) Avoid treating anus, urogenital tract, perineum and genitalia; if the tumor is close to these structures, typically less than 50% volume of the anus and vulva should receive 30 Gy; less than 50% volume of the testis should receive 3 Gy, if the patient prefers to reserve fertility;

c) Avoid treating the lung, through use of appropriate shielding and treatment planning; less than 20% of the lungs should receive 20 Gy (V20);

d) Avoid dose maximums in areas where surgical scars will be placed; this may require reviewing treatment plans with the surgeon;

e) If possible, avoid treating skin over areas commonly traumatized to full dose (e.g., the elbow, knee, shin, femoral neck).

f) If possible, less than 50% of any joints (including shoulder, elbow and knee) should receive 50 Gy.

g) Less than 50% of kidney volumes should receive 18 Gy.

h) No more than 50% of normal weight-bearing bone within the radiation field should receive 50 Gy except when the tumor invades the bone or when there is circumferential involvement of the tumor more than a quarter of the bone or when the bone will be resected in a subsequent surgical resection after radiation.

For any other normal tissue structures, no radiation dose more than the established TD5/5 limit should be given.

5.7.7 I**mage Guidance and Verification Devices**

Portal films or conebeam CT images should be used for target localization and alignment. Daily cone beam CT will be preferred and dose from daily cone beam should be included

in the final dosimetry.

5.7.8 **Simultaneous Integrated Boost**

A simultaneous integrated boost to a total dose of 57.5 Gy in 25 fractions is allowed for patients in whom the surgeon feels a margin negative resection will be difficult to achieve. The boost volume is defined as the high-risk retroperitoneal margin jointly identified by the surgeon and treating radiation oncologist. In this scenario, no further postoperative boost is allowed.

5.7.9 **Postoperative Radiotherapy Boost**

An External Beam or Brachytherapy boost can be given at the discretion of the radiation oncologist. If deemed necessary, the boost volume would include the positive tumor margin (residual tumor) plus a margin of 1 cm. Boost treatment should be delivered within 2 weeks following surgery or after adequate wound healing has occurred.

The target volume for postoperative radiotherapy will be the residual tumor bed as defined by the surgical and pathological findings.

5.7.10 **External Beam Radiotherapy**

Postoperative external beam boost dose is 16 Gy in 8 fractions (once a day) for microscopic disease and 20 Gy in 10 fractions for gross residual disease providing normal tissue is spared (likely requires a biologic or synthetic tissue spacer).

5.7.11 **Anticipated Radiation Therapy Adverse Events**

Acute: Wound complications of any grade are expected to develop in about one third of patients. Other common radiation adverse events include: fatigue, regional alopecia, diarrhea, skin erythema and desquamation within the treatment fields, and reduction in blood counts.

Long-term: Common long-term treatment adverse events include: lymphedema of the extremity receiving radiation and surgery, subcutaneous fibrosis, and joint stiffness. Much less common radiation adverse events include bowel injury, osteoradionecrosis, and bony fracture in the radiation field. There also is a risk of secondary malignancy occurring in the irradiated field.

5.8 General Concomitant Medication and Supportive Care Guidelines

Patients should receive full supportive care, including transfusions of blood and blood products, antibiotics, etc., when appropriate. The reason(s) for treatment, dosage, and the dates of treatment should be recorded.

Throughout the study, investigators may prescribe any concomitant medications or treatments deemed necessary to provide adequate supportive care. All prescription and nonprescription concomitant medication administered up to 28 days prior to enrollment, on an ongoing basis at enrollment, as well as changes in such concomitant medication, and any new concomitant medication taken while the subject is on study, should be recorded up to 30 days after the last dose of ascorbate.

5.8.1 **Antiemetics**. Use Zofran (ondansetron) with caution as this medication may be less effective when used with ascorbate. Prefer alternative agents such as prochlorperazine

5.8.2 **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.

5.8.3 **For diabetics**, be aware high-dose ascorbate can provide false results for finger-stick glucose readings. This effect is dose dependent but can last up to 6h post-infusion. Serum/plasma tests are not affected.[39]

5.9 Criteria for removal from study

Treatment with pharmacological ascorbate may be discontinued if any one of the following criteria is met:

- Intercurrent illness that prevents further administration of treatment,
- Unacceptable adverse event(s),
- Patient decides to withdraw from the study, or
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- Progressive disease as determined by the investigator
- A female subject becomes pregnant or fails to use 2 highly effective methods of contraception (for those subjects who are able to conceive).
- A female subject breast feeds while on study treatment.
- A male subject fails to use a highly effective method of contraception

5.10 Duration of Follow Up

Post-operatively subjects should be followed either by clinic visit or phone contact every 12 ± 4 weeks for approximately 2 years from the end of enrollment, to assess for survival, and disease recurrence. Surveillance imaging and clinical assessment to assess for disease recurrence will be as per NCCN guidelines.

6. DOSING DELAYS/DOSE MODIFICATIONS/ DOSE LIMITING TOXICITIES

6.1 Radiation Therapy

6.1.1 **Dose modification** - Radiation therapy dose will not be modified.

6.1.2 **Treatment delay** - Will only occur for reasons of safety due to intercurrent illness or medical conditions that in the judgment of the radiation oncologist forbid delivery of radiation therapy.

6.1.3 **Interruptions** or delays of greater than three days should be discussed with the study's principal investigator and documented

6.2 Ascorbate Therapy

6.2.1 **Dose reductions** – As per Table 1 (below) if there is a DLT (Section 5.6)

Table 1. Phase 1b Dose De-escalation Schedule

Number of Patients with DLT at a Given Ascorbate Dose	**Decision Rule**
≥ 2	Dose level will be declared toxic. If this is the lowest dose level, stop the study due to excessive toxicity; otherwise enter *three* additional patients at the next lowest dose level.
≤ 1 out of 3	Enter *three* more patients at this dose level.
≤ 1 out of 6	This is the recommended dose for the subsequent Phase2.

Ascorbate Dose Levels

Dose 0	Ascorbate 75 gm IV three times weekly concurrently with radiation
Dose -1	Ascorbate 62.5 gm IV three times weekly concurrently with radiation

- Initial dose for all = Ascorbate 75gm IV

6.2.2 **Test/retest**. If an unexpected adverse event is observed, the treating physicians may withhold ascorbic acid for up to 2 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.

6.2.3 **Dose delays**. 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.

6.3 Dose Limiting Toxicities

Definitions of dose-limiting toxicity:
Toxicity will be evaluated according to CTCAE version 5.0. DLT will be defined as any of the following ascorbate related toxicity or related to the combination of pharmacologic ascorbate and radiation therapy during treatment and up to 28 days after the first pharmacologic ascorbate infusion:

- Infection grade 4
- SAE per 21CFR312.21 with an attribution of *possible, probable, or definite* to ascorbate
- Grade 3 diarrhea or nausea/vomiting despite maximal support
- Any grade 3/4 or greater hematologic or non-hematologic toxicity not explainable by another cause in the opinion of the principal investigator, with the exceptions of:
    - Expected radiation related skin toxicity of any grade.
    - Brief (< 1 week) grade 3 fatigue.

If unexpected DLT occurs, ascorbate administration should be delayed until the DLT has resolved to at least CTCAE version 5.0 grade 1 or baseline and future dose be reduced to dose level -1 as per table 1. If dosing is delayed by more than 4 weeks due to the occurrence of unexpected adverse event or if a patient already dose reduced to level -1 (62.5 gm/dose) experiences another DLT that is considered related to ascorbate, then the subject should be taken off study.

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

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). This study has been assigned as a **risk level 4** as it utilizes an investigator sponsored IND.

7.1 Determination of Reporting Requirements

The clinical research team is responsible for collecting and recording the research data. As these results are collected, all toxicities and adverse events will be identified and reported to the principal investigator (PI). The principal investigator (PI) will determine final relationship of the event to the investigational product (ascorbate):

- Grade 1 and 2 events do not require attributions assigned.
- Grade 3 and higher adverse events require attribution assigned to ascorbate.

Toxicity will be graded according to NCI's Common Toxicity Criteria (CTCAE v5). The principal investigator will have final responsibility for determining the attribution of toxicity as it is related to the investigational product.

7.2 Institutional Review Board reporting requirements

Adverse events that meet criteria of both *serious* and *attributed* (possible, probable, or definite) to the study agent. Thus:

- Serious adverse events *only*
- Attributable to ascorbate (i.e., drug related)
- Report to the IRB *via* HawkIRB within 10 business days of event

7.3 FDA reporting requirements [M.Milhem, sponsor]

Adverse events meeting the criteria of *serious, unexpected,* and *attributed* (possible, probable, or definite) to ascorbate must be reported by the sponsor or the sponsor's appointed designee to the FDA:

- Serious adverse events *only* [21 CFR 312.32]
- Attributable to ascorbate with a causal relationship (possible, probable, or definite)
- Unexpected, as defined by the FDA: "…not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application, as amended."

To help the sponsor meet these reporting requirements, all serious adverse events that are unexpected and attributed to study drug must be reported to the sponsor within 1 business day. This coincides with SAE reporting requirements for the DSMC.

7.4 Routine Adverse Event Reporting Requirements to DSMC

An adverse event (AE) is defined in the *CTEP, NCI Guidelines* [2005] as "any unfavorable and unintended sign (including a clinically significant 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)."

Routine adverse events are captured in OnCore, the clinical trials management system, for review and assessment by the P.I., sponsor, medical monitor, and DSMC.

7.5 Serious Adverse Event Reporting to DSMC

For any experience or condition meeting the definition of a serious adverse event (SAE; 21CFR312.32), from the first day of study treatment and typically continue through the 30 day follow-up period after treatment is discontinued.

Investigators must report to the DSMC any SAE, regardless of attribution to study drug. SAEs must be reported *via* an OnCore SAE Report within 1 business day of learning of the event.

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

- Death
- A life-threatening adverse event
- An adverse event that results in inpatient hospitalization OR prolongation of existing hospitalization for ≥ 24 hours
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- 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. (21 CFR 312.32; ICH E2A and ICH E6).

8. PHARMACEUTICAL INFORMATION – ASCORBIC ACID INJECTION, ASCOR L 500®

8.1 Availability

IND 137968 allows ascorbate to be obtained through McGuff Pharmaceuticals (Santa Ana, CA). Their product Ascor L 500® is now a commercially available injection available in sterile single-use 50 mL vials containing 25 g of ascorbic acid (500 mg/mL).

8.2 Compatibility

Ascorbate is considered a CYP4503A4 inhibitor.[40] 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).

8.3 Storage and Stability

Unopened vials of 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.

8.4 Toxicities

9. 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), AND HYPERTENSION THAT MAY REQUIRE MEDICATION HAVE BEEN REPORTED. CORRELATIVE AND SPECIAL STUDIES

9.1 ROS Research blood

Research blood for future use will be drawn at:

- Baseline prior to test dose of ascorbate
- Prior to XRT dose 1
- Week 3 of radiation therapy prior to XRT dose
- After last fraction of radiation therapy (≤60 minutes of completing that fraction)
- End of study visit which will be ~ 1 month of completing ascorbate therapy

The specimen should be placed on ice immediately after the draw and carried to Spitz lab.

Time point	Ascorbate level (4 cc green top)	ROS (6cc pink)	Immuno (6cc pink)
Test Dose	pre	pre	pre
W1D1	30 min post Ascorbate	pre XRT dose	NA
W3D1	30 min post Ascorbate	pre XRT dose	NA
W5	30 min post last Ascorbate dose	≤ 60 min post last fraction of XRT	30 min post last Ascorbate dose (or can be drawn with ROS after last fraction of XRT)
30 Day post last Ascorbate	NA	yes	yes

9.2 Labile Iron Measurements using MRI Imaging (optional)

Pharmacological ascorbate's mechanism of action is hypothesized to involve changes in labile iron pools within the tumor. The presence of labile iron and/or iron containing metalloproteins is necessary to oxidize ascorbate and eventually lead to the generation of hydrogen peroxide, thereby increasing tumor cell oxidative stress. *In vitro* laboratory studies demonstrate treating GBM and non-small cell lung cancer (NSCLC) cells with high doses of ascorbate increases labile iron pools.[30] Additionally, chelating (e.g., removing) iron from tissue culture media reduces pharmacological ascorbate toxicity to tumor cells.

MRI imaging will be done only for extremity sarcoma subjects prior to ascorbate treatment as a pre-scan control (at diagnosis) and will be done at 60 minutes +/- 30 minutes)and 4 hours post ascorbate treatment (in radiation oncology suite during week 3) as it has been seen that ascorbate levels tend to peak between 1 and 2 hours after injection.[30] Imaging studies will be conducted on

a GE 1.5T/3T MRI scanner using appropriate anatomical coils. Images will be acquired using a T2* fast gradient-echo, multi-echo pulse sequence with short echo time (~6 ms) and repetition time ~25 ms which has shown to be beneficial in previous studies involving iron overload measurements in the liver and heart.[41,42] Image analysis will initially be done using a region of interest approach in which the tumor region will be contoured and then analyzed using a mono-exponential decay of the T2* relaxation time. This image analysis has been shown by *Positano, et. al* [42] to be clinically acceptable and more clinically relevant. Serum iron, transferrin saturation, total iron binding capacity and ferritin levels will be obtained to evaluate if they correlate with responses and T2* imaging findings.

This series will be done at three time points – baseline (as part of simulation in radiation oncology suite or as part of diagnostic MRI if it is being done), week 3 and end of study (as part of diagnosis). The imaging in week 3, done in the radiation oncology suite, is centered around that day's ascorbate infusion. Contrast is not allowed for these sequences and will not be ordered. The following MRI should be ordered to be completed on the same day:

9.2.1 **Contrast**. No contrast is to be used (oral or IV)

9.2.2 **Scheduling**. The infusional T2* is scheduled in week 3. The other time points are at RT simulation and end of treatment study (diagnostic)

9.2.3 **Pre-infusion (week 3).** A pre-infusion T2* MR series will be obtained. This will contain both the T2* and also axial imaging for reference.

9.2.4 **Post-infusion (week 3).** 30 to 90 minutes within the end-of-infusion, a second T2* / axial series will be obtained. This should be obtained prior to the day's radiation therapy.

9.2.5 **4h post-infusion (week 3).** The last T2* / axial series will be done approximately 4h after the end-of-infusion (± 60 minutes).

9.3 Diffusion Weighted Imaging (optional)

Addition of functional diffusion-weighted MRI (DW-MRI) to standard anatomic and postcontrast MRI greatly facilitates interpretation of response to therapy[43,44]. After radiation therapy, new enhancement alone can be seen which may be a result of vascular disruption rather than histologic response. Hence should be interpreted carefully. Although currently not a standard clinical trial endpoint, DW-MRI appears to be a promising as a quick, noninvasive tool showing excellent reproducibility for apparent diffusion coefficient measurements, which reflect tumor cellularity and microarchitecture in STS. However, the relationship of these MRI parameter changes with clinical outcomes has not yet been validated. Hence we intend to evaluate this MRI sequence which is currently acquired routinely for imaging brain tumors and stroke. This sequence will be added to the planning MRI acquired in radiation oncology prior to starting radiation therapy treatment and will be compared to the DWI sequence acquired pre-operatively.

9.4 Ascorbate levels

Serum ascorbate levels will be drawn at screening, 30 mins post at week 1 day 1, as well as 30 mins post week 3 Day 1 and 30 min post last infusion in week 5. This is being done to ensure a target serum level of 20 mM is reached during treatment.

9.5 Tumor tissue

A part of the resected tumor (1 x 1 inch) specimen together with the surrounding normal tissue will be testing for immune markers such as CD4, CD8, T regulatory cells. MDSCs and

macrophages. Flash frozen tissue will be subjected to T2 * imaging testing to determine labile iron concentrations in comparison to the surrounding normal tissue. Molecular testing including next generation testing may be performed on the paraffin embedded tissue specimens. Optional pre-treatment tumor biopsies will be taken from approximately 5 subjects total and immune markers will be compared between pre and post treatment tissue in the immunology lab.

10. STUDY CALENDAR

10.1 Study Procedures

	Screen[b]	Treatment[a]					Off treatment and end of study[c]	Follow Up[d]
		Week 1	Week 2	Week 3	Week 4	Week 5 (+ 1wk[j])		
H & P	X	X		X		X		
Tumor biopsy (optional)	X[b]							
Performance status	X						X	X
Clinical MRI/ Staging CT	X						X	X[e]
Pregnancy test[k] (urine)	X							
G6PD	X							
EKG	X							
CBC w/diff	X			X		X		
PT/INR, PTT & uric acid	X							
Height	X							
Weight, BSA	X						X	
Vitals (BP, pulse)[m]	X	X	x	X	x	X	X	
Comprehensive metabolic panel (CMP)	X	X		X		X	X	
Ascorbate infusion	X[i]	XXX	XXX	XXX	XXX	XXX		
Blood for ascorbate levels[f]	X	X		X		X		
ROS Research blood[g]	X	X		X		X	X	
Immunology research lab[g]	X					X	X	
Iron Panel including ferritin[h]	X						X	
T2* /QSM and DWI MR imaging sequences (optional)	X			X			X	
Adverse Events &Con Meds			X	X	X	X	X	

a study day 1 is defined as radiation therapy day 1 (i.e., fraction 1).

b screening labs, history, and physical must be obtained ≤ 28d from d1. G6PD may be obtained at any time prior to test dose. MRI/CT obtained ≤ 28d from d1 (RT sim is acceptable). Pregnancy test per institutional guidelines. Optional tumor biopsy can be done anytime before week 1 dosing.

c includes both off-treatment evaluation and study completion. Study completion requires a final study dictated visit 4 to 6 weeks after the last dose of ascorbate. Follow at least monthly until resolution of all ascorbate related adverse events.

d approx every 3 months (every 12 weeks [+/- 4 weeks]) for overall survival through passive chart review, phone call or scheduled follow-up visit for 2 years

e Imaging as per standard of care for site specific sarcomas

f 4cc green-top Na-Heparin tube. Draw pre-infusion at 15g dose, then 30 min post-infusion: week 1 day 1 , week 3 and last infusion of week 5

g 6cc pink-top EDTA tube. Draw pre infusion The specimen should be placed on ice immediately after draw and carried to the lab

h At time of screening and end of study

i Test dose of 15 g intravenously once G6PD levels confirmed to be adequate. A physician must review the patient's medication list against the list of medications prohibited on this trial prior to the test dose of ascorbate.

j In case of radiation treatment delays due to holidays

k ±7 days prior to day 1

m Blood pressure should be obtained before and after (≤ 15 min EOI) ascorbate infusion

10.2 Screening Procedures

Procedures to be completed within 28 days prior to first treatment (day 1) (unless otherwise noted).

- Informed consent
- Review of eligibility criteria-
- Recording of medical history and concomitant medications
- Physical exam (including weight)
- ECG
- Vital signs (blood pressure, temperature, pulse and respirations)
- Radiographic imaging (chest, abdomen, and pelvis and all other sites of disease)
- Imaging of primary tumor site

Laboratory Assessments

Procedures to be completed within 28 days prior to enrollment

- CBC w/diff
- CMP and uric acid
- PT/INR, PTT., Urine pregnancy test for females of childbearing potential within 7 days of W1D1

10.3 Post-treatment Evaluations

Following surgical resection of primary tumor, patients will be followed as per NCCN guidelines for approximately 2 years for survival data and disease recurrence.

11. MEASUREMENT OF EFFECT

11.1 Pathologic Response

4 to 6 weeks after the end of neoadjuvant therapy, patients will undergo resection of the treated tumor. The SOC pretreatment biopsy and all the resected tumor samples will be reviewed by the same pathologist.

The percentage of post treatment tumor necrosis must be documented. The primary end point for this study is pathologic complete response (pCR), and is defined as ≥ 95% tumor necrosis following concurrent radiation therapy and ascorbate

Treatment effect on the resected overlying skin tissue will be graded and recorded (to be done by the pathologist). This will be compared with historical controls.

Additional immunohistochemical studies will be performed as determined by the pathologist.

11.2 Time to Disease progression (TTP)

TTP is defined as the time from Enrollment until objective tumor progression including local and distant recurrences.

11.3 Other Response Parameters

The following response assessment guidelines may be amended during the course of the study if changes are made to the current RECIST Guidelines or if a new disease assessment tool becomes available.

Response Measurement Criteria:

Measurements of the tumor in its largest dimension should be obtained at baseline and at the end of the treatment phase, prior to surgical resection.

Response criteria should be by RECIST v 1.1.

- Complete response is the disappearance of all target lesions.
- Partial response is a 30% decrease in the sum of the longest dimensions of the target lesions, relative to baseline.
- Progressive disease is an increase of 20% or more in the sum of the longest dimension of target lesions
- Stable disease is a decrease in the tumor size of < 30% or an increase of < 20%.
- Antitumor response by pathologic assessment will be performed on the resected tumors. Pathologic assessment will be performed per institutional guidelines.
- The percent of tumor necrosis and the percent of tumor-infiltrating lymphocytes will be documented.
- Complete pathological response will be considered ≥ 95% pathologic tumor necrosis.

Radiologic response 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. STATISTICAL CONSIDERATIONS

12.1 Phase Ib

The primary objective of the Phase Ib portion of this study is to confirm the current standard dose of intravenous ascorbate, 75 gm, utilized in patients diagnosed with pancreatic cancer also receiving concurrent chemo radiation is safe within this population of sarcoma patients being treated concurrently with pre-operative radiation. Up to six (6) patients will be recruited to the standard dose, 75 grams administered intravenously 3 times a week during radiation therapy (5 weeks). If at most 1 out of 6 patients experience a DLT, the standard dose will be considered safe. If 2 or more patients experience a DLT, the next cohort of patients will be assigned to dose level -1. The recommended phase II dose (RP2D) will be defined as the highest dose level for which at most 1 out of 6 patients experience a DLT.

12.2 Phase II

The primary objective of the Phase II portion of this study is to evaluate the proportion of patients with pathologic tumor necrosis ≥95%. Previous literature indicates approximately 10% of patients will achieve ≥95% tumor necrosis with pre-operative radiation therapy alone. A sample size of 25 patients achieves 81% power to detect a difference of 20% (from 10% to 30%) using a one-sided binomial exact test. The target significance level is 0.05 and the achieved level is 0.03. If 6 or more patients achieve ≥95% tumor necrosis, the treatment will be deemed worthy of further investigation. For secondary objectives, time to disease progression and overall survival will be estimated using the Kaplan-Meier method. Estimated survival probabilities will be plotted and medians reported, along with 95% confidence intervals. To identify differences in wound complication and Grade 3-4 dermatitis rates relative to historical controls, binomial point estimates and 95% exact confidence intervals will be reported. Mixed effects regression models will be used to estimate changes in labile iron and diffusion imaging sequences on MRI pre and post treatment.

12.3 Sample Size/Accrual Rate

The Phase I portion of this study is expected to require 6 evaluable patients (min 6, max 12). Patients treated in Phase Ib at the RP2D will also be included in the Phase II portion. Additional evaluable patients will be accrued for a total of 25 patients treated at the RP2D. The anticipated accrual rate is 2 patients per month who would meet inclusion criteria.

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PROTOCOL APPENDIX A: Performance status criteria

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

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

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			
isradipine	itraconazole*	ivacaftor	ixabepilone
ketamine	ketoconazole*	lansoprazole	lapatinib
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	

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UIHC Drug Information Center: 1/15

APPENDIX D: DATA AND SAFETY MONITORING PLAN

Type of Clinical Trial:

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

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:

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

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 time the subject signs the informed consent document and continue through the 30-day follow-up period after the last dose of study drug.

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).

Study Stopping and Pausing Rules
The study will pause enrollment and stop treatment for all participants if any of the following events occur pending submission to Regulatory Agencies and review by the DSMC and IRB who will determine if the study may resume:

- Any event of treatment related death in a study subject

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

Subject Registration

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

- The subject's IRB approved (version date) consent form and the date of their consent.
- 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)

All subject registration information is expected to be entered into OnCore within **2 (two) business days** after the subject's study visit.

Subject Data

For HCCC investigator initiated trials, research staff are responsible for entering subject study data (data collection) into OnCore electronic case report forms (eCRFs). These eCRFs must be approved by the PI and statistician prior to study activation to ensure sufficient and necessary data acquisition. All information entered into eCRFs will be traceable to the source documents which are generally maintained in the subject's file.

eCRF data entry needs to be timely and should be entered into OnCore as soon as possible but no later than **14 (fourteen) business days** after the subject's visit, including adverse events, tumor measurements, administration of study medication, concomitant medications, labs, and vitals. Physical exam assessments must be entered no later than **14 (fourteen) business days** following completion of the physician's clinic note in the medical record.

Timely data entry facilitates remote monitoring of data, allows the data to progress appropriately through the data cleaning process, and helps prevent a backlog of data queries.

Forms Monitoring

OnCore eCRF data are monitored on a routine basis (dependent on accrual) to ensure all data are entered completely, accurately, and within time requirements outlined above. The assigned DSMC monitor will coordinate and complete the data monitoring review. When the time comes to monitor a study (based on patient accrual and assigned risk level of trial) the monitor arranges for a selection of cases to be reviewed from among the subjects registered in OnCore. As part of the forms monitoring process, the assigned monitor will issue queries via OnCore (linked to the eCRF) to resolve missing, incomplete, and/or incorrect information. A member of the research team is expected to respond to these monitoring queries within **14 (fourteen) business days.**

The monitoring process can often identify misunderstandings or deficiencies in the written, research protocol requirements earlier in the study process and thereby improve data quality and

reduce rework.

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.