A First-in-Human Clinical Trial of Pharmacologic Ascorbate and Ferumoxytol Combined with Concomitant Temozolomide and External Beam Radiation Therapy for Newly Diagnosed Glioblastoma

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SUMMARY OF CHANGES FROM PREVIOUS VERSION

Affected Section(s)	Summary of Revisions Made	Rationale
Cover	Updated date & Amendment	Housekeeping
Heading	Updated date & amendment	Housekeeping
1.4	Deleted ROS research sample	Housekeeping; left in by error from A3
1.4	Deleted C2-6 ASC levels	Housekeeping; left in by error from A3
1.4	Updated footnote "d' to align to protocol	Housekeeping
1.4	Deleted footnote "e"	Housekeeping; left in by error from A3
1.4	Updated footnote "j" to align to protocol	Housekeeping
1.4	Revised footnote "k" to reflect protocol dictated imaging required at 3 to 4 months post RT	Due to variability in clinical imaging.
1.5	Inserted table for research & pK samples	Clarification
8.1.3	Inserted ascorbate level after FMX infusion 4	Oversight in A3
8.1.3	Inserted text that research iron levels can be omitted if clinical are drawn within window	Clarification
8.1.3	Inserted research iron to be drawn at T2* after FMX infusion 2	Oversight in A3
8.1.4	Revised text for protocol dictated diagnostic MRI	Due to variable clinical nature, only require protocol imaging at 3 to 4 months timepoint.
8.2.3	Inserted "Clinical," for ferritin & iron panel	Clarification
8.2.4	Inserted, "Draw 2 weeks after last infusion in ferumoxytol period."	Oversight in A3
8.2.4.1	Deleted ascorbate level	Housekeeping; left in by error from A3

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

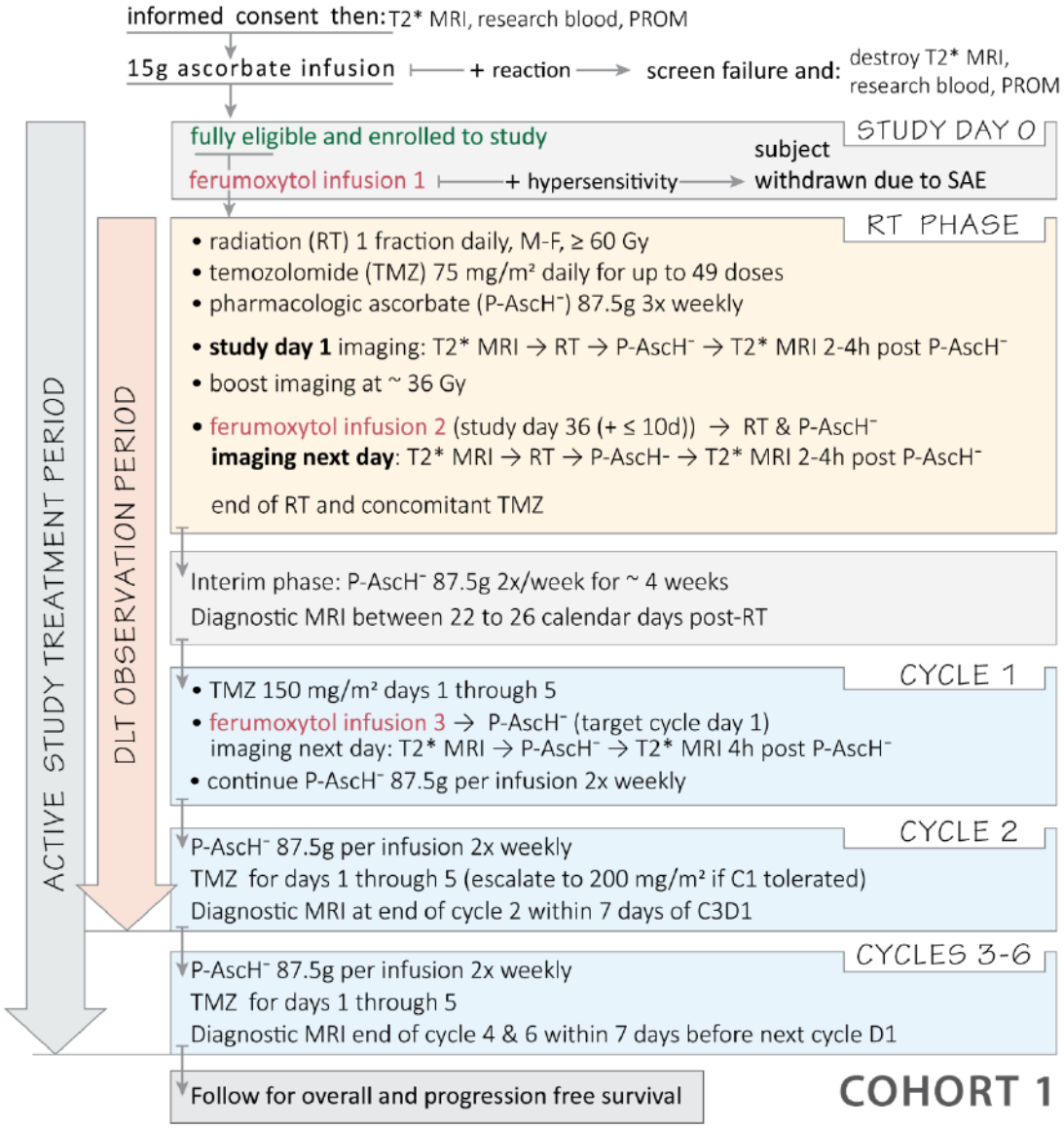
The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; a determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 PROTOCOL SUMMARY

1.1. SYNOPSIS

Title:	A First-in-Human Clinical Trial of Pharmacologic Ascorbate and Ferumoxytol Combined with Concomitant Temozolomide and External Beam Radiation Therapy for Newly Diagnosed Glioblastoma.
Study Description:	A phase 1, first-in-human study to evaluate the safety of up to two ferumoxytol dosing schedules when given concomitantly with pharmacologic ascorbate, temozolomide, and radiation for the treatment of newly diagnosed glioblastoma (GBM).
Objectives:	Primary objective. Using traditional dose-limiting rules, determine the safe dosing schedule for ferumoxytol: twice every five weeks or once every five weeks. Secondary objectives. Estimate progression free survival (PFS), overall survival (OS), objective response rate (ORR), as well as evaluate and characterize adverse events per CTCAE version 5.
Endpoints:	Dose limiting toxicities (DLTs) Adverse events, graded using CTCAE v. 5 Tumor measurements, using RANO Objective response rates, using RANO T2* relaxation time (ms) and QSM tissue susceptibility (ppb) Patient reported outcome measures (PROMs)
Study Population:	Patients with newly diagnosed glioblastoma referred for temozolomide and radiation therapy for initial treatment of their malignancy.
Phase:	Phase 1 first in human
Description of Enrollment Sites:	Enrollment will be opened at a single academic site in the midwestern United States.
Description of Study Agent / Intervention:	**Ferumoxytol**. 510 mg / infusion. Dosing frequency as per cohort (Cohort 1: once per treatment period; Cohort 2: twice per treatment period). **Pharmacologic ascorbate**. 87.5 g / infusion. 3x weekly during RT phase, 2x weekly during adjuvant phase (up to 6 cycles). **Temozolomide**. Concomitant with radiation: 75 mg/m^2 oral once daily. Adjuvant: 150 mg/m^2 oral once daily, days 1 through 5 of 28-day cycle. Dose escalation to 200 mg/m^2 in Cycle 2-6 if tolerated. **External beam radiation therapy**. Radiation administered at 1.8 Gy/fraction, one fraction/day, for a total of 34 fractions. Administer on business days only.
Study Duration:	Primary endpoint is estimated to be completed within 2 years.
Participant Duration:	Active study treatment for 9 months, active follow-up for 2 years.

1.2. SCHEMA: COHORT 1 [STARTING COHORT]



Screening. Physical exam, medical history, recommendation for combined modality therapy, and concomitant medication review for prohibited medications. Potential participants must not have a history of Steven's Johnson syndrome, hemochromatosis, allergy to ferumoxytol, or G6PD-deficiency.

Solicited AEs. Nausea, vomiting, diarrhea, fatigue, skin condition (screen against rash and itching) and pain (note location of pain, if any).

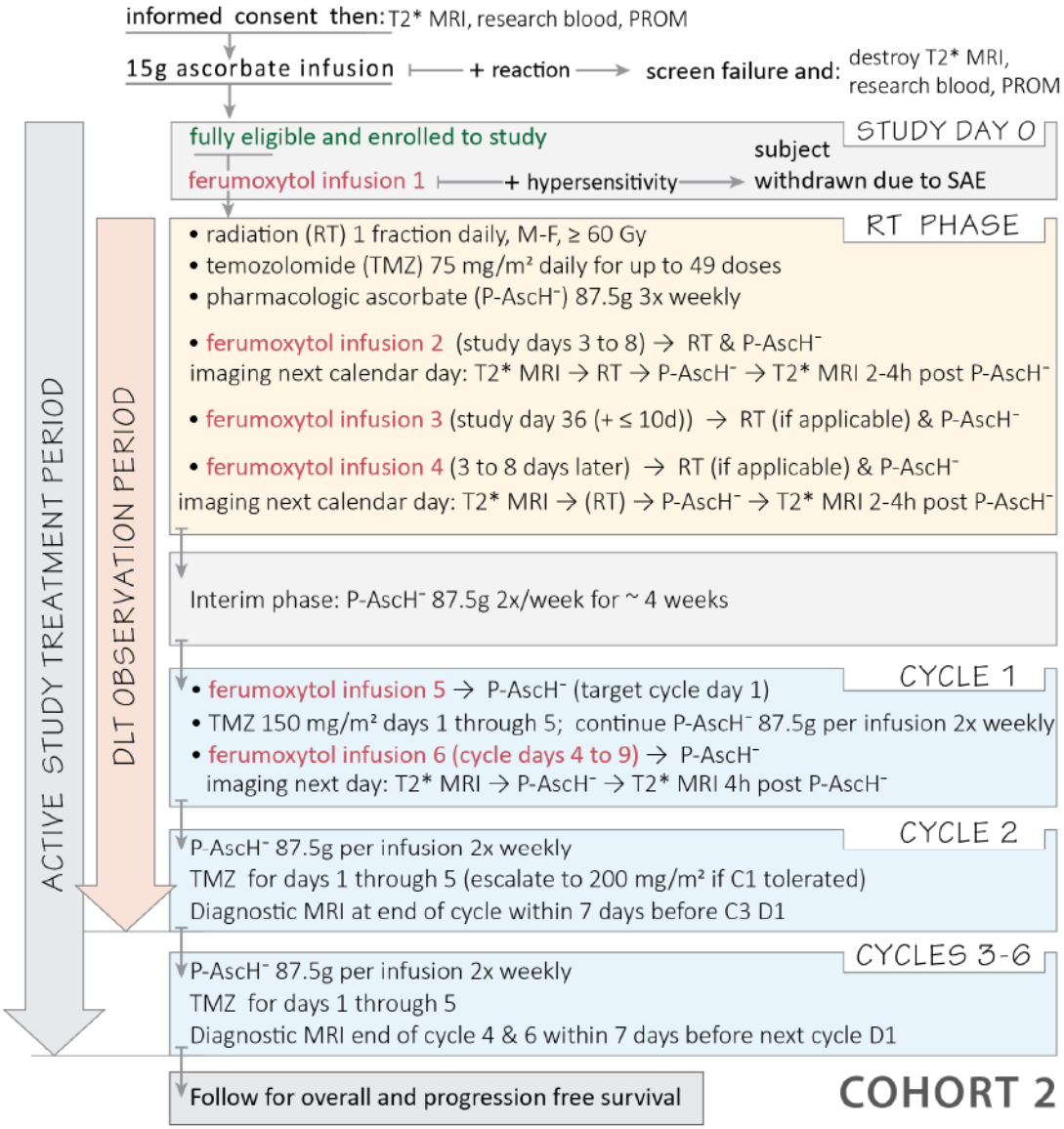
PROM. Fit-for-purpose questions to collect patient experience data on key symptoms: fatigue, nausea/vomiting, headaches, and strength/weakness.

Critical endpoints. Dose limiting toxicities through end of cycle 2 day 28 (C2D28). Tumor response assessment via RANO criteria through end of treatment.

End of study treatment. Completion of 6 cycles of adjuvant therapy (through C6D28), dose limiting toxicity, disease progression, unacceptable event, non-compliance, or withdrawal.

Follow-up. 30d follow up and then chart review for 2 years to assess for long-term radiation sequelae, progression free survival, and overall survival.

1.3. SCHEMA: COHORT 2 [ESCALATION COHORT]



Screening. Physical exam, medical history, recommendation for combined modality therapy, and concomitant medication review for prohibited medications. Potential participants must not have a history of Steven's Johnson syndrome, hemochromatosis, allergy to ferumoxytol, or G6PD-deficiency.

Solicited AEs. Nausea, vomiting, diarrhea, fatigue, skin condition (screen against rash and itching) and pain (note location of pain, if any).

PROM. Individualized questions based on subject preference and individual risk assessment (e.g. weight loss, fatigue, pain).

Critical endpoints. Dose limiting toxicities through end of cycle 2 day 28 (C2D28). Tumor response assessment *via* RANO criteria through end of treatment.

End of study treatment. Completion of 6 cycles of adjuvant therapy (through C6D28), dose limiting toxicity, disease progression, unacceptable event, non-compliance, or withdrawal.

Follow-up. 30d follow up and then chart review for 2 years to assess for long-term radiation sequelae, progression free survival, and overall survival.

1.4. SCHEDULE OF ACTIVITIES (SOA)

Procedures	BASE	day 0[a]	RT	Interim	C1	C2-6	*b*
Informed consent	SCR						
Medical history	X						
G6PD	SCR						
PT / INR	≤21d						
Uric Acid	≤21d						
ECG	≤21d						
Concomitant med review	SCR	X	weekly		*c*	*c*	x
Pre-infusion ascorbate level	≤21d						
Post-infusion ascorbate level			*d*		d22-28		
Research iron / ferritin draw			*d*		*d*		
Physical exam with NANO	≤21d		weekly		*c*	*c*	x
Performance status	X		weekly		*c*	*c*	x
Pregnancy test	SCR						
CBC w/diff	≤21d		weekly		*f*	*f*	x
CMP	≤21d		*g*		*g*	*g*	x
AST, ALT, TBIL, ALP		*h*	*h*		*h*		
Clinical iron panel & ferritin	≤21d		*h, j*		*h, j*	*j*	x
Diagnostic MRI	RT sim					*k*	
T2* imaging	*m*	*m*	*m*		*m*		
Ferumoxytol infusions		*h, n*	*h, n*		*h, n*		
Ascorbate infusions[p]	15g		3x / wk	2x / wk	2x / wk	2x / wk	
Blood pressure	*p*		*p*	*p*	*p*	*p*	
Temozolomide			x		d1-d5	d1-d5	
Radiation therapy			x				
Patient reported outcomes	r		weekly		*c*	*c*	x
Solicited adverse events	*s*		weekly		*c*	*c*	x
Review for unsolicited AEs		Protocol section 8.2.4.2					

a Day zero (d0) is defined as the day of the first ferumoxytol infusion. It is the calendar day prior to RT fraction 1

b 30 day follow-up, preferably before subsequent therapy. Sections 8.1 and 8.2

c Can be ≤ 1 calendar week before D1; does not need to be repeated if TMZ is delayed

d **Research labs for pk/pD/imaging**: See section 1.5. Only ASC levels are drawn at first 87.5g ASC infusion, for the cohort-dependent MR imaging timepoints (protocol section 8.1.3.1) and last ascorbate infusion during RT. Obtain research iron samples prior to any treatment that day for T2* imaging days and obtain the ASC levels ≤ 15 min EOI.

f d22 (± 3 calendar days) and up to 72 hours from temozolomide dosing

g At discretion of treating medical oncologist

h **Hold and call for safety assessment prior to ferumoxytol infusion**. Retest if hemolyzed. May be drawn up to 72 hours prior to planned ferumoxytol infusion. Results must be reviewed by delegated physician prior to ferumoxytol infusion.

j **Order clinical iron panel** 2 weeks (± 3 days) after the last FMX infusion of the treatment period and repeat in 2 weeks if transferrin saturation of ≥ 90% and/or ferritin > 1000 ng/ml. Order if subject notes right upper quadrant discomfort.

k Protocol dictated diagnostic imaging at 3 to 4 months after completing radiation therapy. Obtain copies of other MRI obtained clinically and assess for RANO.

m Baseline T2* MR must be done prior to 15g test dose. If participant is a screen fail, images must be destroyed. Subsequent dependent upon cohort. Refer to schema & section 8.1.3.1

n 30 minutes between ferumoxytol and other infusions. Refer to protocol section 6.1.2.1.

p BP is performed prior to day's infusion and then ≤ 15 minutes of end of infusion

r Prior to 15 g test dose; must be completed in paper so that it is destroyed if participant is a screen fail.

s Query for adverse events of special interest

1.5. PK & RESEARCH SAMPLES

Timepoint / Milestone	**Ascorbate levels** (4 mL NaHep tube)	**Clinical Iron & Ferritin**	**Research Iron & Ferritin**	**Immunology** (optional; 4mL EDTA)
15g ascorbate test dose (SCR)	Before	—	—	Before
Ferumoxytol infusion 1 (Day 0)	—	≤ 72h; hold & call	—	—
1st 87.5g infusion during RT	Post ASC (≤ 15 min if possible)	—	—	—
Ferumoxytol infusion 2	Post ASC (≤ 15 min if possible)	2 weeks post; repeat if elevated§	≤ 72h of infusion, then, Prior to any treatment on T2 MRI day	—
Ferumoxytol infusion 3	—	≤ 72h; hold & call	—	—
Ferumoxytol infusion 4	Post ASC (≤ 15 min if possible)	2 weeks post; repeat if elevated§	≤ 72h of infusion, then, Prior to any treatment on T2 MRI day	—
Last 87.5g infusion during RT	Post (≤ 15 min if possible)	—	—	—
Ferumoxytol infusion 5	—	≤ 72h; hold & call	—	—
Cycle 1 Day 1 temozolomide	—	—	—	Prior to any treatment (including TMZ)
Cycle 1 Days 22 through 28	Post, 1x	—	—	—
Ferumoxytol infusion 6	Post ASC (≤ 15 min if possible)	2 weeks post; repeat if elevated§	≤ 72h of infusion, then, Prior to any treatment on T2 MRI day	—
Cycle 1 Day 8 (± 1 day)	—	—	—	Anytime
30 day follow-up		X		
§ retest clinically every 2 weeks if transferrin saturation ≥90% and/or ferritin > 1, 000 ng/ml)				

2. INTRODUCTION

2.1. STUDY RATIONALE

Despite aggressive multimodality therapy including surgery, radiation, and chemotherapy, 5 year overall survival for GBM ranges from zero to a scant 14%.[1] Over time, the majority of patients will experience a recurrence ultimately leading to death. Preliminary studies demonstrate pharmacologic ascorbate induces cytotoxicity in GBM cells; initial studies have assessed the safety of adding pharmacologic ascorbate to chemotherapy and chemoradiation and identified recommended phase 2 doses.[2-8]

Preclinical work suggests that ascorbate's anti-cancer efficacy is related to available redox active iron content[2,9]; thus supplementing GBM iron stores *via* ferumoxytol may increase ascorbate mediated GBM cell killing further enhancing tumor response to radiation and temozolomide. Ferumoxytol is FDA-approved for iron-deficiency anemia; this trial seeks to determine the safety of adding ferumoxytol and pharmacological ascorbate to the standard of care regimen for newly diagnosed GBM.

2.2. BACKGROUND

2.2.1. HIGH GRADE GLIOMA AND CURRENT THERAPY

GBM is the most common primary brain tumor in adults. Diagnosed in over 14,000 Americans per year, GBMs are highly aggressive, diffusely infiltrative tumors of the cerebral hemispheres.[10,11] Occurring primarily in adults over age 40, these malignancies are rapidly progressive and almost uniformly fatal. Despite the current standard of care, GBM median survival ranges from 14 to 16 months. [12-16]

Current standard therapy recommendations for GBM include surgical resection, when feasible, followed by radiation therapy and temozolomide. Of all of the treatment modalities, radiation therapy has the greatest effect upon survival.[17,18] When added postoperatively, radiation boosts survival from 14 weeks with surgery alone to 36 weeks with adjuvant radiotherapy.[19] The current standard of care calls for approximately 60 Gy delivered to the enhancing tumor and edema in 1.8 or 2.0 Gy fractions (61.2 Gy in 34 fractions or 60 Gy in 30 fractions) with concurrent temozolomide.[16] Temozolomide is an oral cytotoxic alkylating agent, which has recently been added to the standard of care for high grade glioma. A 2005 EORTC study of concomitant and sequential temozolomide with radiation therapy demonstrated significantly improved overall survival with temozolomide (14.6 months vs. 12.1 months) over radiation alone,[16] with little increase in toxicity.[20] The 2-year survival rate also increased from 10.4% to 26.5%.[16] Tumor-treating fields (TTF) delivers electric fields to GBM disrupting mitosis and causing apoptosis.[21] A randomized trial showed that TTF added to standard of care improved median PFS from 4.0 to 6.7 months and overall survival from 16.0 to 20.9 months.[21] However, many GBM patients prefer not to wear TTF transducer arrays for 18 hours per day on their scalp and elect not to receive TTF therapy.

Despite these advances, however, treatment options for GBM remain largely palliative. At five years, the overall survival for GBM ranges from zero to a scant 14%.[1] Over time, the majority of patients will experience a recurrence that will ultimately lead to death. Thus, additional relatively non-toxic therapies which enhance the efficacy of temozolomide and radiation are needed to improve long term survival.

2.2.2. PHARMACOLOGIC ASCORBATE AS AN ANTI-TUMOR AGENT

The evidence for use of ascorbate in cancer treatment falls into two categories:

- clinical data on dose concentration relationships
- 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.[22] As doses exceed 200 mg, absorption decreases, urine excretion increases and ascorbate bioavailability is reduced.[22,23] 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.[24] 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.[25] 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. Ascorbate mediated cancer cell death was dependent on the formation of hydrogen peroxide (H_2O_2).[26] H_2O_2 generation is dependent upon ascorbate concentration, incubation time, and the presence of redox active metals; [H_2O_2] generation has a linear association with [ascorbate] and increases as a quadratic function of ascorbate radical. Ascorbate acts an electron donor to O_2 to form superoxide and, eventually, H_2O_2.

When ascorbate is infused intravenously the resulting pharmacologic concentration distributes rapidly into the extracellular water space.[27] *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.[28] 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.[22] Additionally, many studies have found that ascorbate readily crosses the blood brain barrier and results in concentrations up to 20x that of the plasma concentration.[29-31]

Ascorbate-mediated cell death is due to H_2O_2 generation, *via* ascorbate radical formation, with ascorbate serving as an electron donor.[25,28] In the presence of redox active metals, such as ferric iron (Fe^{3+}), ascorbate is oxidized and the metal is reduced, for example ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}). The reduced metal then donates an electron to molecular oxygen forming superoxide ($O_2^{\bullet-}$). $O_2^{\bullet-}$ is then converted into H_2O_2 via superoxide dismutases. Hydrogen peroxide can then interact with redox active metals through Fenton chemistry to from hydroxyl radicals ($OH^{\bullet}$) which cause DNA, lipid, and protein damage culminating in cell death. In contrast, the same pharmacologic ascorbate concentrations in whole blood and non-malignant tissues generate little detectable ascorbate radical and no detectable H_2O_2.[32] This can be accounted for by the expression of iron regulatory proteins that limit cellular redox active iron pools as well as efficient and redundant H_2O_2 catabolic pathways in whole blood and non-malignant tissues relative to those in media or cancer cells.

2.2.3. NCT 01049880: A PHASE 1 STUDY OF P-ASCH- IN SOC THERAPY FOR GBM

A phase I study of adding ascorbate to standard therapy based on Stupp et al[16] was initiated in 2013 [NCT 01049880, Buatti PI].[33] Subjects were assigned to high-dose ascorbate dose cohorts for the concomitant portion of therapy. Dose cohorts ranged from 15 g to 87.5 g. During the adjuvant phase, subjects underwent ascorbate dose escalations to achieve a targeted plasma ascorbate level of 350 mg/dL. Subjects received high-dose ascorbate during the adjuvant phase for up to 6 temozolomide cycles.

Treatment was well-tolerated with minimal adverse events. No subject experienced a grade 3 or greater hematologic or non-hematologic toxicity. Summary tables for hematologic and non-hematologic (Table 1) adverse events are provided with comparator values from the Stupp trial.[3,16] Adverse events associated with pharmacological ascorbate were grade 1 dry mouth, grade 1 chills, and grade 2 hypertension. Dry mouth was transient and ended shortly after

Table 2. Adverse events from the phase 1 GBM trial (NCT01752491) compared to historical control

	Radiation Therapy Phase				Adjuvant Phase			
	TMZ + RT + Asc (*n* = 13)		TMZ + RT Stupp *et al.* (*n* = 284)		TMZ + Asc (*n* = 12)		TMZ Stupp *et al.* (*n* = 223)	
Adverse Event	**Grade 2**	**Grade 3**	**Grade 2**	**Grade 3/4**	**Grade 2**	**Grade 3**	**Grade 2**	**Grade 3/4**
White blood cell decreased	4 (31%)	0 (0%)	not reported	7 (2%)	1 (8%)	1 (8%)	not reported	11 (5%)
Neutrophils decreased	1 (8%)	0 (0%)		12 (4%)	0 (0%)	1 (8%)		9 (4%)
Platelet count decreased	0 (0%)	0 (0%)		9 (3%)	1 (8%)	0 (0%)		24 (11%)
Anemia	0 (0%)	0 (0%)		1 (<1%)	0 (0%)	0 (0%)		2 (1%)
Fatigue	3 (23%)	1 (8%)	74 (26%)	19 (7%)	2 (15%)	0 (0%)	73 (25%)	18 (6%)
Rash	0 (0%)	0 (0%)	26 (9%)	4 (1%)	0 (0%)	0 (0%)	13 (5%)	5 (2%)
Infection	1 (8%)	0 (0%)	3 (1%)	9 (3%)	0 (0%)	0 (0%)	6 (2%)	12 (5%)
Vision	0 (0%)	0 (0%)	39 (14%)	3 (1%)	0 (0%)	0 (0%)	28 (10%)	2 (<1%)
Nausea	5 (38%)	1 (8%)	38 (13%)	2 (<1%)	4 (31%)	0 (0%)	52 (18%)	4 (1%)
Vomiting	0 (0%)	2 (15%)			1 (8%)	0 (0%)		
The most common adverse events in the ascorbate phase 1 trial were dry mouth (grade 1, 61-75%, grade 2 8%) and chills (grade 1, 50-53%) and were deemed related to ascorbate infusion. These events resolved within 1h post-infusion.								

infusion. Grade 1 chills were infusion related, most likely due to the infusion of prepared ascorbate that had been stored at 4˚C. Grade 2 hypertension occurred in some subjects during infusion, most likely due to the volume of hypertonic fluid infused.

2.2.4. ASCORBATE INDUCED GBM T2* IMAGING CHANGES

We previously identified that GBM cells have increased levels of labile redox active labile iron compared to normal human astrocytes[2] and treatment with increasing doses of ascorbate increase GBM labile iron pools with minimal change in normal human astrocyte labile iron pools (**Figure 1A**). Western blot analysis demonstrates greater expression of the transferrin receptor in GBM cells compared to normal human astrocytes (**Figure 1B**). Increasing labile pools by treating with ferrous ammonium sulfate increased cell sensitivity to ascorbate (**Figures 1C & 1D**).[2]

Paramagnetic iron (Fe^{3+}) catalyzes ascorbate's oxidation to form superoxide and diamagnetic iron (Fe^{2+}). Because Fe^{3+} shortens MR imaging relaxation times, quantitative MR imaging using T2* and quantitative susceptibility mapping (QSM) can detect ascorbate induced changes in iron oxidation state. In an on-going phase 2 clinical trial evaluating the efficacy of pharmacological ascorbate in combination with standard of care in treating newly diagnosed GBM patients (NCT02344355),[34] after radiation fraction number 20, subjects received quantitative MR

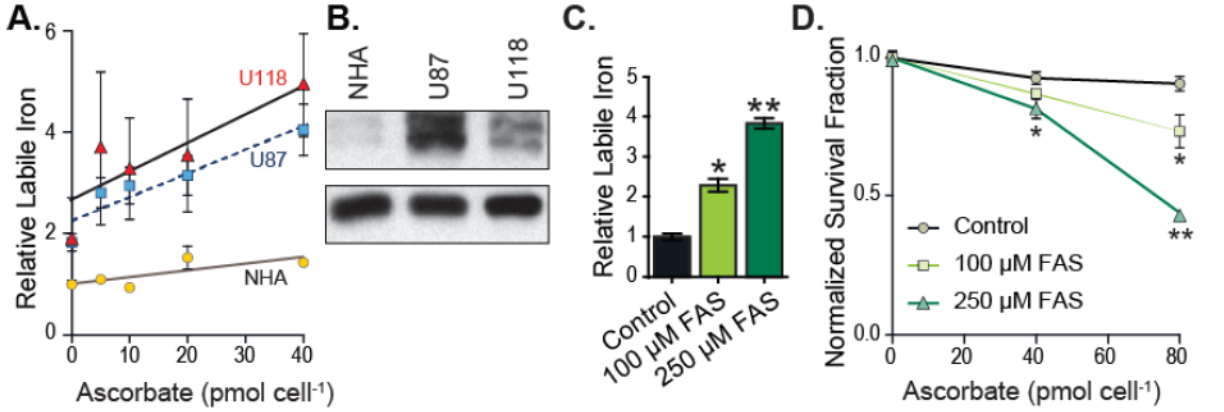


Figure 1: GBM cells have increased labile iron pools which mediates ascorbate sensitivity. (**A**) Relative labile iron pools were assessed at baseline and with increasing ascorbate concentrations by calcein assay. NHA had reduced labile iron pools relative to GBM cells (U118 and U87). Exposure to ascorbate increased labile iron pools in GBM cell lines to a greater extent than NHA cells. (**B**) Western blot analysis of transferrin receptor (upper panel) in NHA and GBM cell lines (U87 and U118). Actin is the loading control (bottom panel). (**C**) Exposure of NHA to increasing concentrations of ferrous ammonium sulfate (FAS) increases the relative labile iron pool. (**D**) Increasing NHA labile iron pool increases sensitivity to ascorbate toxicity by clonogenic survival assay. * represent significant difference from other groups, at least $p < 0.05$.

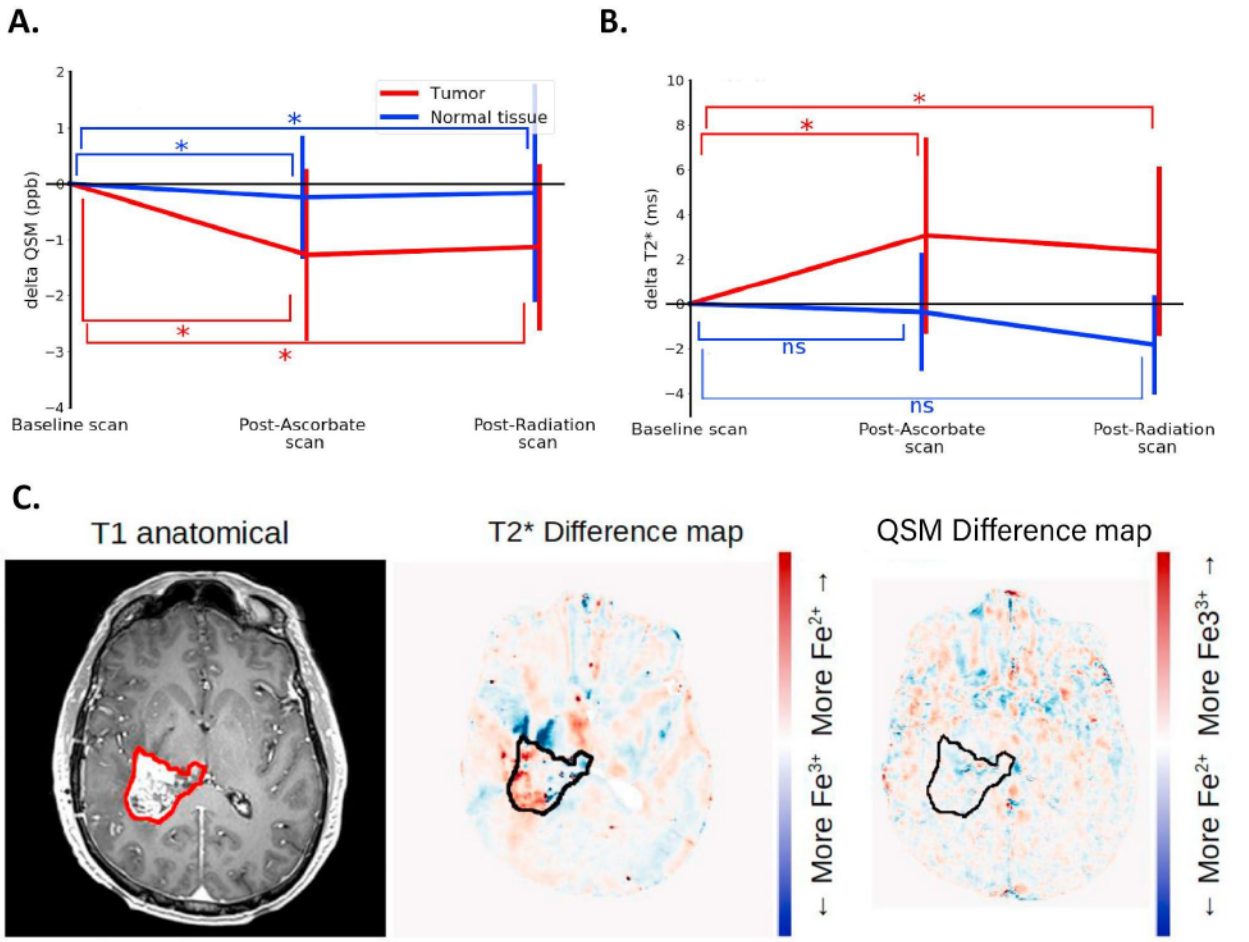


Fig. 2. (A). Differences between mean changes in QSM observed in the contrast enhancing region of tumors and normal tissue. Contrast enhancing region of tumors were contoured on a contrast-enhanced T1 image by a board-certified radiation oncologist. Data are normalized per subject to the baseline scan. **(B)** Differences between mean changes in T2* observed in contrast enhancing region of tumors and normal tissue. Time-course of mean T2* values in the contrast enhancing region of tumors and normal tissue of subjects receiving P-AscH- show a persistent change in tumors post P-AscH **(C)** Representative images of T2* and QSM change as a result of acute P-AscH- administration. **LEFT.** T1 contrast enhanced anatomical image showing the extent of the contrast enhancing region of tumor (contoured in red). **CENTER**. Difference map (post P-AscH- minus pre P-AscH-) showing the change in T2* as a result of acute P-AscH-administration. Red indicates an increase in T2*, consistent with Fe3+ being reduced to Fe2+. **RIGHT.** Difference map (post P-AscH- minus pre P-AscH-) showing the change in magnetic susceptibility as a result of acute P-AscH- administration. Blue indicates a decrease in magnetic susceptibility, consistent with Fe3+ being reduced to Fe2+.

imaging prior to ascorbate infusion, 3-4 hours post ascorbate infusion, and then 3-4 hours post radiation treatment. Two MRI methods, T2* and quantitative susceptibility mapping (QSM) have been shown to be sensitive to total iron concentrations.[34] QSM assesses the phase of the MR signal to estimate the magnetic susceptibility of tissue while T2* assesses the magnitude of the MR signal.[34] Tissues with increased paramagnetic metals, particularly Fe^{3+}, will have strong magnetic moments that can be detected by QSM and T2*. Our previous work identified that Fe^{3+} has a greater magnetic susceptibility leading to substantially shorter relaxation times than Fe^{2+}.[34] The ascorbate mediated conversion of Fe^{3+} to Fe^{2+} within a tumor is hypothesized to result in longer T2* relaxation and lower QSM times. Evaluation of the first 15 subjects that participated in the phase 2 GBM study demonstrated significantly reduced QSM (**Figure 2A**) and prolonged T2* relaxation times in GBM tumors following ascorbate infusion.[34] Normal tissue analysis demonstrated minimal to no change in QSM or T2* relaxation times following ascorbate pharmacological administration (**Figures 2A and B**). A representative subject demonstrating change in T2* imaging is shown (**Figure 2C**).

Evaluation of the T2* imaging at baseline (prior to ascorbate treatment, radiation, and chemotherapy) suggests initial iron redox state may serve as a marker for progression free survival. Analysis of the first 23 subjects that had a short T2* relaxation rate (≤ 53.5 ms) had a median PFS of 11.6 months while subjects with a long T2* relaxation

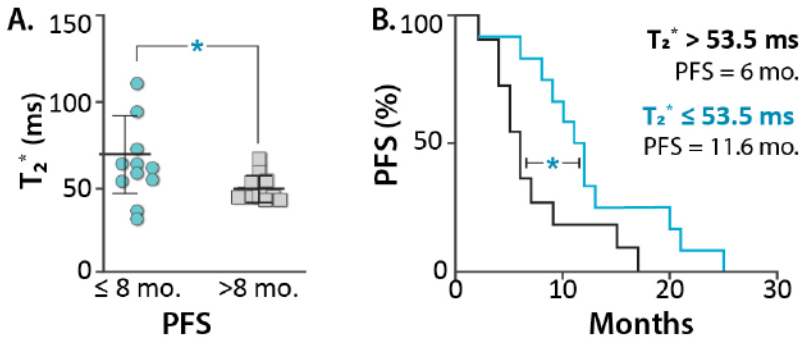


Figure 3: GBM tumors with shorter relaxation times are more sensitive to P-AscH⁻. Treatment naïve human GBM subjects (n = 23) received T2* MRI scans prior to the beginning of P-AscH⁻, TMZ, and radiation. **A.** Mean T2* relaxation time in patients stratified by median cohort PFS (8 mos.). *p<0.05 using a paired, two-tailed Student's T-test. **B.** Kaplan-Meyer plot of patients on study stratified by median cohort T2* relaxation time (53.5 ms). *p<0.05 using a Log-Rank (Mantel-Cox) test.

rate (> 53.5 ms) had a median progression free survival of 6 months (**Figure 3**). The shorter T2* relaxation time suggests the GBM tumor contains more paramagnetic iron (Fe^{3+}) and since Fe^{3+} is needed to oxidize ascorbate, these tumors would be predicted to have a better treatment response. Furthermore, this supports our hypothesis that approaches which increase GBM tumor iron content may improve response to pharmacological ascorbate.

2.2.5. FERUMOXYTOL IN TREATMENT OF IRON DEFICIENCY ANEMIA

Ferumoxytol (Feraheme™) is an ultrasmall (~20 nm) superparamagnetic iron oxide nanoparticle that is coated with a semisynthetic carbohydrate. Each ferumoxytol nanoparticle contains 2000 magnetite molecules (Fe_3O_4). Ferumoxytol was approved by the FDA in 2009 to treat iron deficiency anemia associated with chronic kidney disease and end-stage renal disease.[35-37] The FDA approved dosing regimen to correct iron deficiency anemia is two 510 mg iron infusions 3 to 8 days apart.[35] A phase 2 randomized clinical trial comparing ferumoxytol (510 mg delivered two times 5 ± 3 days apart) and iron sucrose (100 mg for 10 doses delivered within 3 weeks) in treating iron deficiency anemia in patients with chronic kidney disease identified similar adverse event and efficacy data between the two regimens.[38-40] Adverse events with an incidence greater than or equal to five percent included muscle spasms (5%) and nausea (7.5%). Other notable adverse events include anaphylactic reaction (1.3%), hypoglycemia (2.5%), diarrhea (2.5%), constipation (2.5%), urinary tract infection (3.8%), and nasopharyngitis (3.8%).[35,38,39] A phase 3 trial evaluating ferumoxytol vs. placebo for the treatment of iron deficiency anemia in adults with chronic kidney disease with a history of unsatisfactory oral iron therapy using a similar two-dose regimen of 510 mg IV ferumoxytol found ferumoxytol to be effective and well tolerated.[40,41] Treatment related adverse events were similar in incidence between ferumoxytol and placebo and include: headache (5.8%), nausea (4.6%), dizziness (3.9%), diarrhea (2.8%), urinary tract infection (2.8%), nasopharyngitis (2.6%), vomiting (2.1%), fatigue (2.0%), rash (2.0%), abdominal pain (1.8%), arthralgia (1.5%), dyspnea (1.6%), anemia (0.7%). The incidence of serious adverse events was similar between the two groups (2.6% ferumoxytol; 3.0% placebo) with the majority being attributed to comorbid disease. One patient that received ferumoxytol experienced an anaphylactic reaction (0.2%) and three patients that received ferumoxytol developed hypersensitivity (0.5%).

2.2.6. FERUMOXYTOL IN MR IMAGING

Ferumoxytol was first investigated as an MRI contrast agent in 2003.[42] It is currently approved by the FDA as an alternative MRI contrast agent in place of gadolinium for gadolinium allergic reactions or in patients with renal failure.[43-47] Because of ferumoxytol's size and carbohydrate coating, it has a long intravascular residence time of 14-21 hrs with delayed intracellular uptake.[48] In brain lesions, there is slow leakage of ferumoxytol from the blood vessel through the disrupted blood brain barrier.[48] MRI T2 and T2* signal changes peak approximately 24 hrs after ferumoxytol administration.[49] As iron is released locally from ferumoxytol, there is a decreased T2* relaxation time demonstrating intracellular uptake.[48] After ferumoxytol leaves the blood vessel, the iron nanoparticles are taken into macrophages and astrocytes.[50] In the cells, the carbohydrate coating is cleaved and eliminated in the urine or feces while the iron core is incorporated into the cell's iron stores.[48] Because the iron is taken into cells by transferrin, unlike gadolinium, there is no concern for long-term deposition of ferumoxytol in the brain.[51-53] In GBM tumors,

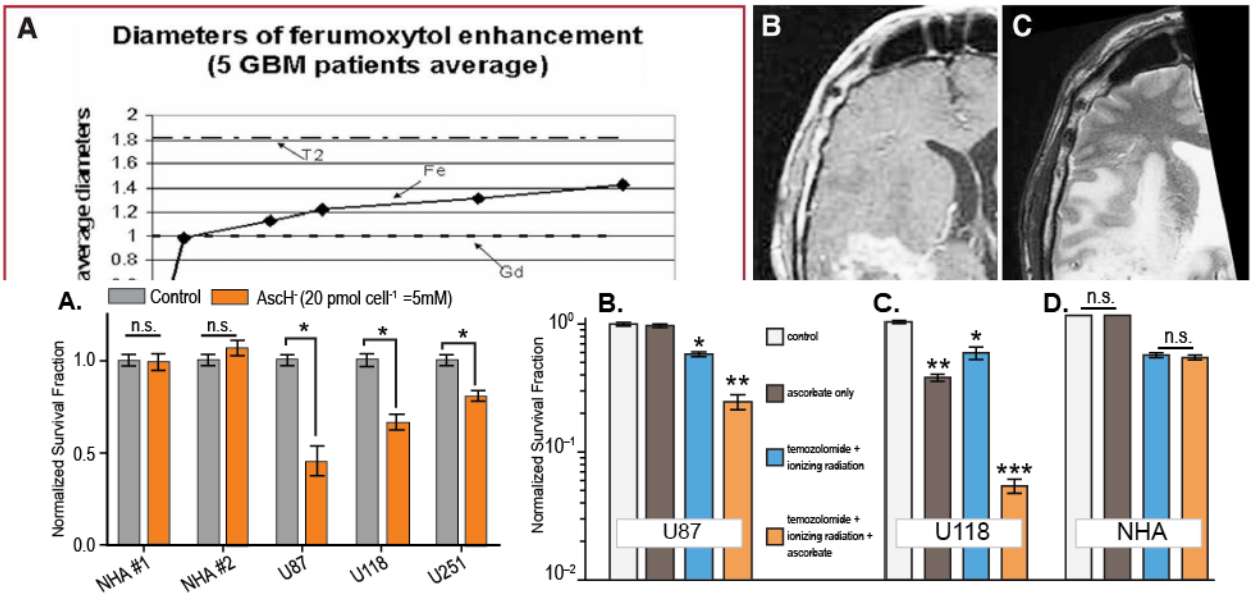


Figure 5: P-AscH⁻ selectively sensitizes GBM cells to radiation and temozolomide therapy. (A) Clonogenic survival post 5mM P-AscH⁻ exposure for 1 hour to normal human astrocytes (NHA) from two individuals and GBM cell lines (U87-MG, U118, and U251). (B-D) Clonogenic survival of U87-MG (B), U118 (C), and NHA (D) following exposure to temozolomide (TMZ), 2 Gy radiation (RT), and 2 mM P-AscH⁻. P-AscH⁻ increased the sensitivity of GBM cells lines (B and C) to radiation and temozolomide but did not enhance the toxicity of temozolomide and radiation in NHA (D). *, **, *** represents significant difference from other groups, at least $p < 0.05$. "n.s." is not statistically significant.

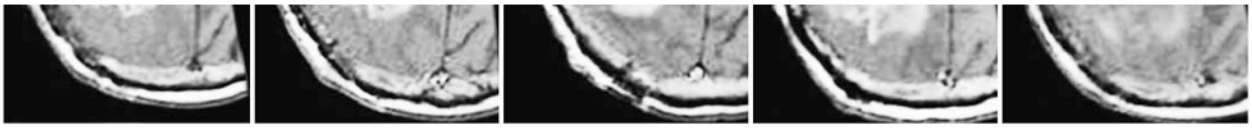


Figure 4. A, line graph showing the extent of ferumoxytol enhancement in a GBM patient. The average diameter changes of ferumoxytol enhancement over time (solid line) are compared with the diameter of Gd enhancement (dashed) and T2-weighted signal abnormalities (dashed dotted). Gd T1-weighted (B), T2-weighted (C), and post-ferumoxytol T1-weighted (D–H) MRI scans at five time points (D, 4–6 h; E, 6–20 h; F, 24–28 h; G, 48–52 h; H, >72 h). Figure from : Neuwelt EA, Várallyay CG, Manninger S, et al. The potential of ferumoxytol nanoparticle magnetic resonance imaging, perfusion, and angiography in central nervous system malignancy: a pilot study. *Neurosurgery*. 2007;60(4):601-612. PMID: 17415196x

ferumoxytol enhancement continues for up to 72 hours slowly expanding out from gadolinium enhanced region but never exceeding the area of increased FLAIR signal (**Figure** 4).[49] Because iron deposition is limited to the areas of the GBM tumor and surrounding edema, areas that are of concern for radiation treatment fields, ferumoxytol may be an ideal imaging and therapy agent when combined with pharmacological ascorbate.

2.2.7. IN VITRO FINDINGS

Previous studies demonstrated that pharmacological doses of ascorbate can kill cancer cells while normal cells are relatively unaffected.[54] **Figure 5** demonstrates pharmacological ascorbate enhances sensitivity of GBM cell lines, U87 and U118 to radiation and temozolomide but not normal human astrocytes (NHA). Cells were treated with ascorbate (0.5 mM) for one hour followed by exposure to 2 Gy of radiation and 25 μM temozolomide. Cell survival was determined by clonogenic cell survival assay. These results support the hypothesis that ascorbate enhances radiation and temozolomide induced cell killing of GBM cells and not NHA cells.

2.2.8. IN VIVO FINDINGS

To develop an orthotopic *in vivo* model to investigate GBM redox active labile iron, mice bearing orthotopic GBM tumors were assessed by T2* imaging daily for 5 days followed by the collection of GBM and normal brain tissue for labile iron pool measurement by EPR spectroscopy (**Figure 6**). As seen in the *in vitro* studies (**Figure 1**), murine orthotopic GBM tumors had shortened T2* relaxation times compared to contralateral normal brain tissue (**Figure 6A**). Analysis of the labile iron pool content in GBM tumors and contralateral normal brain tissue by EPR spectroscopy showed increased redox active iron in the GBM tumors (**Figure 6B**).

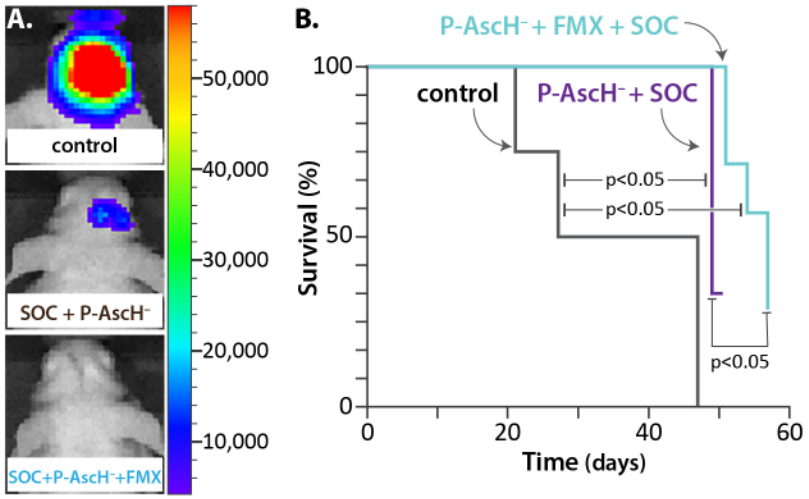


Figure 7: Addition of FMX to P-AscH⁻ enhances SOC therapy in an orthotopic glioma model. A. Luciferized U87 tumors were implanted orthotopically and confirmed 6 d later using bioluminescent imaging (BLI). Mice were given FMX (10 mg kg^{-1}) 24 h prior to the beginning of treatment. Mice then received either SOC (weekly 2.5 mg kg^{-1} TMZ + 6 Gy x 2 Fx whole brain radiation) or SOC + P-AscH⁻ (daily 4 g kg^{-1}). Mice received 2 subsequent cycles of FMX, P-AscH⁻, and/or TMZ following RT. Tumor growth was monitored using BLI for 14 d in the adjuvant radiation phase. Representative BLI at 14 d following completion of radiation. **B.** Ongoing Kaplan-Meyer curve comparing overall survival of control (untreated), SOC + P-AscH, and SOC + P-AscH + FMX

To assess if ferumoxytol improved the efficacy of P-AscH⁻ combined with ionizing radiation and temozolomide, mice bearing orthotopic luciferized U87 tumors were treated with standard of care [(radiation 12 Gy/2 fx), temozolomide (2.5 mg kg^{-1} weekly)] ± pharmacological ascorbate (4 g kg^{-1} daily) ± ferumoxytol (10 mg kg^{-1} weekly) for 21 days. Tumor volumes were monitored using bioluminescent imaging 14 days into the adjuvant radiation phase (**Figure 7A**). Tumor bearing mice treated with the combination of standard of care + pharmacological ascorbate + ferumoxytol had reduced tumor burden compared to mice receiving only standard of care + pharmacological ascorbate (**Figure 7B**). No differences in animal behavior or weights were observed with no significant toxicities associated with the addition of ferumoxytol. Thus, ferumoxytol appears to safely enhance GBM anti-tumor response to pharmacological ascorbate, radiation, and temozolomide in a preclinical model.

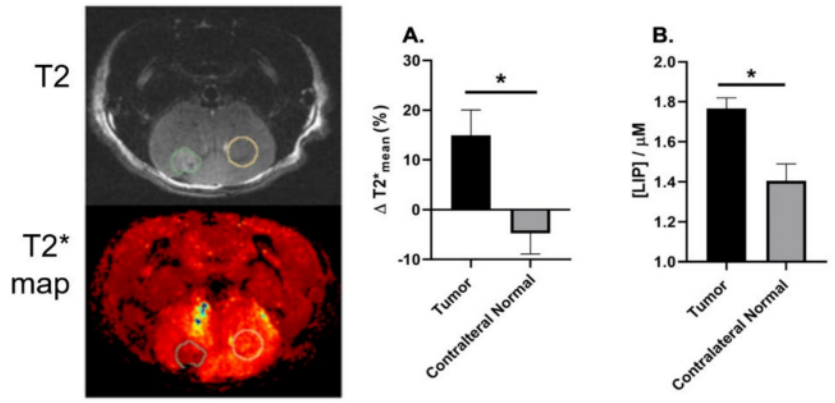


Figure 6: Analysis of redox active iron content in an orthotopic GBM mouse model. U87 tumors were implanted orthotopically and confirmed 10 d later using T2 MR imaging. **A**. Following confirmation of GBM growth, mice were imaged daily for 5 days with T2* imaging. GBM tumors demonstrated increased T2* relaxation times compared to contralateral normal brain tissue. **B**. After 5 days of growth, GBM and contralateral normal brain tissue was harvested and labile iron pools [LIP] measured by EPR spectroscopy. GBM's had increased labile iron pools compared to contralateral normal brain tissue.

2.3. RISK/BENEFIT ASSESSMENT

2.3.1. KNOWN POTENTIAL RISKS

In addition to risks of standard of care treatment for newly diagnosed glioblastoma (i.e., maximum safe resection followed by radiation and temozolomide), the investigational treatment has known adverse reactions from the published literature and prescribing information as approved by the U.S. FDA.

Ferumoxytol is most notable for three rare but serious adverse reactions: hypersensitivity including anaphylaxis, hypotension, and iron overload. Other adverse events associated with ferumoxytol are consistent with the standard treatment for GBM: diarrhea, nausea, headache, fatigue. Pharmacologic ascorbate is associated with dry mouth, transient blood pressure elevations, and urolithiasis, including kidney stones. A significant risk from pharmacologic ascorbate is the false elevation of finger-stick or urine-dip glucose tests within 12 hours post-infusion. A phase 1 trial seeking a safe dosing frequency is needed to establish which dose limiting toxicities present the most significant risk.

2.3.2. KNOWN POTENTIAL BENEFITS

Initial work suggests an increase in treatment efficacy of FMX combined with ascorbate and SOC therapy as well anecdotal subject feedback of feeling better with the ascorbate infusions. Known benefit is associated with participating in a clinical trial likely associated with patient focused medical care and attention to treatment detail.

2.3.3. ASSESSMENT OF POTENTIAL RISKS AND BENEFITS

Treatment response for those diagnosed with GBM remains abysmal. While promising as an adjuvant therapy, pharmacologic ascorbate may be enhanced by increasing the available GBM labile iron during treatment. This is possible by exploiting an FDA-approved drug with an established toxicity profile in an adult population. The availability of safety data combining ferumoxytol and pharmacological ascorbate with standard anti-cancer therapies is minimal. Some subjects participating in pharmacologic ascorbate trials received ferumoxytol infusions for the treatment of cancer-associated anemia. To date, a serious adverse event has not occurred in these few subjects. Steps to reduce risk include regular and repeated laboratory testing, weekly assessments, immediate access to trained healthcare personnel, and securing compliance to protocol. Given the poor outcomes associated with GBM, the risks and benefits are equitable for this study.

3. OBJECTIVES AND ENDPOINTS

Objective	Endpoint	Justification
Primary		
Determine the recommended phase 2 dose of ferumoxytol during treatment with pharmacologic ascorbate, radiation therapy, and temozolomide for newly diagnosed GBM.	Dose Limiting Toxicities	A rule-based design using pre-defined dose limiting toxicities (i.e., adverse reactions that are deemed clinically unacceptable despite benefit) is easiest and safest to implement in this Phase 1 study.[55]

Secondary		
Objective	**Endpoint**	**Justification**
Estimate progression free survival (PFS) as defined by the RANO criteria.	Tumor measurements and clinical assessments as per RANO criteria.[56,57] Progression must be unequivocal. Radiation simulation MR / CT is used as baseline. PFS includes death as well as progression.	• Preferred regulatory endpoint.[58] • Better correlate to overall survival.[58] • Can serve as surrogate endpoint for accelerated approval, unlike time-to-progression. [58]
Evaluate overall survival.	From day 1 of therapy to death from any cause in the intent-to-treatment population.	• Easily measured.[58] • Objective measurement.[58] • Provides clear survival benefit outcome.[58]
Estimate initial effect size utilizing objective response rate (ORR) as defined by RANO criteria CR/PR.	Complete response and partial response are defined using RANO criteria.[56,57] (Stable disease is not considered a component of the objective response rate, because it can reflect natural disease history rather than a direct therapeutic effect).	• Considered to measure an effect attributable to the drug and not natural history of the disease. [58] • Objective response rate is considered appropriate for accelerated or regular drug approval and is approved by FDA for single-arm studies with smaller sample sizes. [58]
Describe tumor size and clinical response over time (as per RANO criteria).[56,57]	RANO[56,57] disease response (CR, PR, SD, PD) beginning 12 weeks after the last radiation treatment using radiation simulation MRI/CI as the baseline.	• Tumor measurements are considered more clinically relevant than other biomarkers. [58] • Tumor measurements support both accelerated traditional drug approval. [58]
Evaluate and characterize adverse events.	Clinician adverse event assessment using CTCAE v.5	• Safety assessment.[58] • Initial data to investigate ameliorating effects. [58] • Provides critical data to inform patient-focused drug development.[59]
Exploratory		
Explore the relationship between changes in T2*/QSM imaging, disease response, and overall survival.	T2* relaxation time (ms) QSM tissue susceptibility (ppb) RANO response[56,57] Overall survival	• Initial step in identifying a correlative predictive measure undergoing combined therapy.[58] • Imaging based biomarkers are considered preferable to blood/tissue biomarkers. [58]
Explore the patient experience patient-reported outcome measures in this clinical trial.	Individualized patient outcome measures, with individual questions obtained from validated item database (PROMIS, EORTC, PRO-CTCAE).	• Initial step in determining impact in holistic health for patients undergoing combined therapy.[58] • Quality of Life and patient reported outcomes (symptoms) are discrete entities; comparison between QoL and symptoms will provide exploratory insight for patient focused drug development • Patient focused drug development is required per FDA.[60-62]

4. STUDY DESIGN

4.1. OVERALL DESIGN

Hypothesis
Ferumoxytol and pharmacologic ascorbate are safe when administered during concomitant radiation therapy and temozolomide for newly diagnosed glioblastoma (GBM).

Phase
Phase 1 design.

Description of trial
Single site, non-randomized, open-label clinical trial employing convenience-based sampling.

Methods to minimize bias
The following strategies are employed to minimize bias:

- All patients referred for initial treatment will be screened against eligibility.
- Subject eligibility is reviewed by an independent data and safety monitoring committee.
- Adverse events and dose limiting toxicities are reviewed by an independent data and safety monitoring committee.
- Radiologic evaluation for RANO will be performed with a blinded evaluator.

Dose escalation
Described in section 6.1.2, *dosing and administration*.

Study groups and arms
The phase 1 assessment explores 2 dose cohorts.

Interventions

- Ferumoxytol (Feraheme®, AMAG Pharmaceuticals)
- Pharmacologic ascorbate (ASCOR®, McGuff Pharmaceuticals, Inc)

Interim analysis
A cohort-based interim analysis for dose-limiting toxicities will be performed after the dose limiting toxicity window for the last participant enrolled per dose cohort (i.e., after every 6 participants) or upon DLT threshold (i.e. 2 DLTs per cohort). Interim analysis is performed by the study statistician and formally reviewed by the data and safety monitoring committee.

Stratifications
None.

Sub-studies and/or sub-analyses
None.

4.2. SCIENTIFIC RATIONALE FOR STUDY DESIGN

The study design is rule based escalation.[55] This design enables dose escalation based upon observed toxicities as well as the experience of other treated participants. The study's design is adapted from the traditional 3+3, which has been a foundational method for conducting phase 1 clinical trials. This design requires no modeling, it is simple to implement, and well known by both researchers and treating healthcare staff. Having multiple participants per experimental cohort allows for interpatient variability while screening for toxicities.[55] The primary criticism of this strategy – involving a potentially excessive number of participants at subtherapeutic and/or potentially toxic doses – is not as a significant concern based upon the FACT clinical trial for ferumoxytol as well as the prior completed work on pharmacologic ascorbate for glioblastoma multiforme.[3,38,40,63]

4.3. JUSTIFICATION FOR DOSE

4.3.1. FERUMOXYTOL

Route of administration. Intravenous, consistent with USPI.[35]

Treatment period. Ferumoxytol treatment periods are 5 calendar weeks, with the first ferumoxytol infusion defining day 0.

Starting dosage and frequency. 510 mg iron administered on study day 0. The second ferumoxytol treatment period occurs 5 weeks after the first. The final ferumoxytol treatment period is synchronized with the first adjuvant temozolomide cycle, beginning cycle day 1.

Planned maximum dosage and frequency. Dependent upon assigned cohort.

Dosage justification. As recommended within the current FDA-approved prescribing information and peer-reviewed literature.[35,37]

Dosing regimens. Up to two regimens will be examined.

- Cohort 1 [starting]: This cohort evaluates one administration of ferumoxytol per 5 week treatment period
- Cohort 2 [escalation]: This cohort evaluates two administrations of ferumoxytol per treatment period

Dosing regimen justification. The ferumoxytol dosing regimens are based upon the prescribing literature as well as the FACT clinical trial which evaluated consecutive 5-wk treatment periods of ferumoxytol for patients diagnosed with chronic kidney disease-associated iron deficiency anemia.[35,37,38,40]

4.3.2. PHARMACOLOGIC ASCORBATE

Route of administration. Intravenous, consistent with USPI.[64]

Starting dosage. 87.5 g in 1250 mL of sterile water for human use.

Maximum dosage. N/A – no dosage adjustments.

Dosing regimen. Regimen is based upon published phase 1 dose seeking and subsequent phase 2 data (RT phase, adjuvant phase).[3,33,63] Ascorbate is restricted to one infusion per calendar day.

- **Radiation (RT) phase**. Three infusions per calendar week. For holiday weeks, inclement weather, or mid-week starts, participants must receive at least 2 infusions during the week.
- **Adjuvant (ADJ) phase**. Beginning the week post-radiation, participants will receive 2 infusions per week through completion of 6 cycles of adjuvant temozolomide chemotherapy.

Justification. Pharmacologic ascorbate dose is based upon a prior phase 1 clinical trial as well as the phase 2 clinical trial currently in active follow-up.[2,3,63]

4.3.3. TEMOZOLOMIDE

Route of administration. Oral, consistent with USPI.[65]

Starting dosage. 75 mg / m^2 concomitant; 150 mg/m^2 for adjuvant cycle 1.

Maximum dosage. No adjustments during concomitant; maximum of 200 mg/m^2 during adjuvant.

Dosing regimen. Regimen is based upon treatment phase (RT phase, adjuvant phase).

- **Radiation (RT) phase**. Once daily for up to 49 consecutive days during concomitant radiation, beginning with the first radiation dose.
- **Adjuvant (ADJ) phase.** Once daily for 5 consecutive days, beginning day 1 of a 28-day cycle. Adjuvant treatment begins no less than 28 days after the last radiation treatment.

Justification. All parameters follow the FDA-approved regimen as per the USPI.[65]

4.3.4. RADIATION THERAPY

Route of administration. External beam, focal radiation therapy.

Starting dosage. 1.8 or 2 Gy/fraction.

Maximum dosage. N/A – no dosage adjustments.

Dosing regimen. One fraction per day, Monday through Friday, for a total dose of 60 or 61.2 Gy in 30 or 34 fractions.

Justification. Minimum radiation dose per NCCN guidelines is 60 Gy to planned target volume.

4.4. END OF STUDY DEFINITION

A subject is considered to have completed the study if one of the following has been met:

- The subject has completed interventions and visits as described in the schedule of activities (section 1.2),
- The subject has withdrawn or been withdrawn from further intervention and has completed the necessary follow-up as prescribed (section 7),
- The study has been terminated and the subject has completed the necessary interventions as indicated by the study sponsor.

If a subject has withdrawn from study treatment, the subject must provide consent to continue to undergo study assessments (even if the subject is withdrawn/withdraws due to an adverse event). This must be documented in the study chart. If a subject declines further follow-up or assessment, per OHRP/DHHS and FDA guidance, no further interaction may take place with the subject in regard to the study.

5. STUDY POPULATION

5.1. INCLUSION CRITERIA

In order to be eligible to participate in this study, an individual must meet all of the following criteria:

1. Willingness and ability to provide informed consent consistent with Good Clinical Practice (i.e., legally authorized representative will not be used / allowed for this study).
2. Stated willingness to comply with all study procedures for the duration of the study.
3. Stated willingness to have a central venous access device (e.g., CVAD, infusion port) placed.
4. Aged 18 years or older.
5. Newly diagnosed (i.e., within 6 weeks), histologically or molecularly confirmed glioblastoma or diffuse midline glioma.
6. Therapy to begin within 6 weeks of last surgery
7. Able to take oral medication
8. ECOG performance status of 0, 1, or 2 (KPS of >50)
9. Medically fit, as determined by prescribing oncologists, to undergo temozolomide and radiation therapy.
10. Agree to use of contraception from screening until at least 90 days after the last study treatment (study participant should not discontinue contraception until discussing with their treating oncologist(s)).
11. Not have significant co-morbid central nervous system disease, such as multiple sclerosis.
12. Agree to Lifestyle Considerations (see section 5.3) throughout study duration]

5.2. EXCLUSION CRITERIA

An individual who meets any of the following criteria will be excluded from participation in this study:

1. Current use of the following drugs and cannot have a drug substitution or decline the drug substitution: warfarin, flecainide, methadone, amphetamines, quinidine, and chlorpropamide. Pharmacologic ascorbate may affect urine acidification and, as a result, may affect clearance rates of these drugs.
2. Current use of antiretroviral drugs (e.g., nelfinavir, abacavir, emtricitabine, lamivudine, stavudine, tenofovir disoproxil fumarate, zidovudine). Pharmacologic ascorbate acid a known CYP450 3A4 inducer, which results in lower serum levels of antiretroviral drugs.
3. Insulin requirement
4. Requires blood glucose monitoring using finger-stick glucose checks.
5. Medical requirement or indication for iron supplementation (including ferumoxytol, ferrous gluconate, ferrous fumarate, or ferrous sulfate). NOTE: Over the counter, patient-elective supplementation is acceptable.
6. Inability to undergo MR imaging.
7. Inability to tolerate a 15g test dose of ascorbate.
8. Pregnancy or lactation (note: potential participants should not engage in 'pump & dump' strategy; lactation must be discontinued).
9. Known allergic reaction to ferumoxytol.
10. History of Steven's Johnson Syndrome
11. History of hemochromatosis.
12. Prior radiation treatment that would result in field overlap. For potential participants who have undergone nuclear medicine therapy, including PRRT, the study's radiation oncologist must approve study entry.
13. G6PD (glucose-6-phosphate dehydrogenase) deficiency
14. Platelet count < 100,000 /mm3 within 21 days of first treatment
15. Creatinine ≥ 1.5x the institutional upper limit of normal within 21 days of the first treatment or if creatinine is elevated a creatinine clearance of < 60 mL/(min 1.73 m^2)
16. Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection (requiring inpatient admission or a delay to start of therapy), symptomatic congestive heart failure, unstable angina

pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

17. Treatment with another investigational drug within 30 days prior to study treatment day 1. Imaging trials (including investigational PET or NM tracers) as well as observational trials are acceptable.
18. Clinical trials with an endpoint of treating the patient's cancer, including behavioral, nutritional, and/or device human subject studies.

5.3. LIFESTYLE CONSIDERATIONS

During this study, participants are asked to:

- Refrain from alcohol use during therapy through cycle 2. Use should be documented in subject's case book.
- Refrain from acetaminophen or acetaminophen containing products through cycle 2. Use should be recorded in subject's case book.
- Refrain from consumption of red wine, Seville oranges, grapefruit or grapefruit juice, [pomelos, exotic citrus fruits, grapefruit hybrids, or fruit juices] from 7 days before the start of study day 0 until after the end of cycle 6. **Note**: If participants consume these, they should be documented in the subject's case book.
- Carry patient safety card / wallet card describing potential ascorbate drug interactions at all times for emergency personnel.
- Use birth control during active study treatment – abstinence is acceptable .

5.4. SCREEN FAILURES

Individuals who do not initially meet the criteria for participation in this trial (screen failure) because of a laboratory value may be rescreened as described below:

- **Pregnancy**. A woman who has recently undergone an abortive procedure may still have elevated hCG levels. In this situation, documentation from one of the treating physicians that the patient is not pregnant will suffice *in lieu* of a pregnancy test.

5.5. STRATEGIES FOR RECRUITMENT AND RETENTION

This phase 1 clinical trial will employ convenience sampling with the following sampling considerations:

- Patients of all genders, races, and ethnicities are invited to participate. Under-represented race and ethnicity will be targeted for a larger phase study evaluating effectiveness.
- Consistent with ICH Good Clinical Practice E6 guidelines, this study will not include patients who cannot provide informed consent, including children, prisoners, and the mentally ill. At this time, there is no proven therapeutic benefit and the study's scientific rationale does not require inclusion of these patient bases. Thus, it is appropriate to delimit participation to only those patients who can understand the study and provide independent informed consent.
- Anticipated accrual rate is 2 subjects every month, based upon historical trends, which indicate that 6 patients meeting criteria are seen quarterly at UIHC.
- Up to 12 subjects will be enrolled at a single center within the midwestern United States.
- Potential participants will be identified through treating oncologists at an academic medical center from an established patient base. The treating oncologist or principal investigator will approach potential subjects, depending upon local/institutional recruitment requirements.
- Public recruitment strategies will not be employed however this study will be listed on ClinicalTrials.gov

- Subjects will receive a thank-you note from the study team after completing radiation therapy and parking compensation. Research staff will maintain contact with subjects to remind about upcoming appointments and determine what would make the visit more pleasant (e.g. heated blankets, music choice, extra pillows).
- As a therapeutic oncology trial of unknown efficacy, subjects are not compensated.

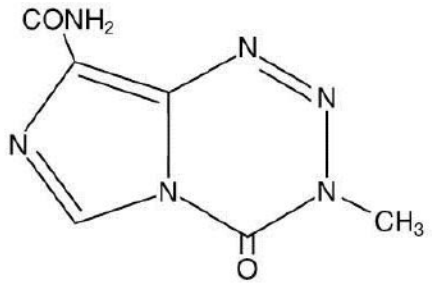
6. STUDY INTERVENTION

6.1. STUDY INTERVENTION(S) ADMINISTRATION

6.1.1. STUDY INTERVENTION DESCRIPTION

6.1.1.1. TEMOZOLOMIDE (TEMODAR®)

From the FDA approved package insert: Temozolomide is an alkylating drug. The chemical name of temozolomide is 3,4-dihydro-3-methyl-4-oxoimidazo[5,1-d]-as-tetrazine8-carboxamide. The structural formula of temozolomide is:



The material is a white to light tan/light pink powder with a molecular formula of C6H6N6O2 and a molecular weight of 194.15. The molecule is stable at acidic pH (<5) and labile at pH >7; hence TEMODAR can be administered orally and intravenously. The prodrug, temozolomide, is rapidly hydrolyzed to the active 5-(3-methyltriazen-1-yl) imidazole-4-carboxamide (MTIC) at neutral and alkaline pH values, with hydrolysis taking place even faster at alkaline pH.

6.1.1.2. PHARMACOLOGIC ASCORBATE (ASCOR®)

From the FDA approved package insert: ASCOR (ascorbic acid injection) for intravenous use is a colorless to pale yellow, preservative-free, hypertonic, sterile, nonpyrogenic solution of ascorbic acid. ASCOR must be diluted with an appropriate infusion solution (e.g. 5% Dextrose Injection, USP, Sterile Water for Injection, USP). The chemical name of Ascorbic Acid is L-ascorbic acid. The molecular formula is C6H8O6. Each ASCOR, 50 mL, Pharmacy Bulk Package vial contains 25,000 mg ascorbic acid, equivalent to 28,125 mg sodium ascorbate. Each mL of ASCOR contains 500 mg of ascorbic acid (equivalent to 562.5 mg of sodium ascorbate which amounts to 65 mg sodium/mL of ASCOR), 0.25 mg of edetate disodium, and water for injection. Sodium hydroxide and sodium bicarbonate are added for pH adjustment (pH range 5.6 to 6.6). It contains no bacteriostatic or antimicrobial agent.

6.1.1.3. FERUMOXYTOL (FERAHEME®)

From the FDA approved package insert: Feraheme is an iron replacement product containing ferumoxytol for intravenous infusion. Ferumoxytol is a non-stoichiometric magnetite (superparamagnetic iron oxide) coated with polyglucose sorbitol carboxymethylether. The overall colloidal particle size is 17-31 nm in diameter. The chemical formula of Feraheme is Fe5874O8752-C11719H18682O9933Na414 with an apparent molecular weight of 750 kDa. Feraheme Injection is a sterile aqueous colloidal product that is formulated with mannitol. It is a black to reddish brown liquid and is provided in single-dose vials containing 510 mg of elemental iron. Each mL of the sterile colloidal solution of Feraheme Injection contains 30 mg of elemental iron, 30 mg polyglucose sorbitol carboxymethylether, and 44 mg of mannitol. The formulation is isotonic with an osmolality of 270-330 mOsm/kg. The product contains no preservatives and has a pH of 6 to 8.

6.1.2. DOSING AND ADMINISTRATION

6.1.2.1. FERUMOXYTOL

Dose. 510 mg iron in 17 mL [FDA approved dose]

Treatment period. Ferumoxytol treatment periods are 5 calendar weeks, with the first ferumoxytol infusion defining day 0.

Treatment period scheduling. Cannot be an overlap between treatment periods that results in a treatment period being less than 5 calendar weeks (i.e., there must be ≥ 5 weeks between the first infusion of each treatment period). A gap of ≤ 10 calendar days may be used between treatment periods for scheduling purposes (e.g., align to office visit or adjuvant cycle day 1). No five week period should have more than two ferumoxytol infusions.

Infusion frequency per treatment period. Dependent upon assigned cohort.

Route of administration. Intravenous.

Hold and call. Liver function tests (ALT, AST, ALP, TBIL) must be drawn, resulted, and reviewed prior to initiating ferumoxytol infusion. Window for draw is within 72 hours before scheduled ferumoxytol infusion.

Initiation. First administration is the calendar day before the first fraction of radiation. The first ferumoxytol infusion defines study day 0. Note: Radiation must begin the next calendar day and required a minimum of 2 consecutive fractions. **Thus, day 0 must be on a Monday, Tuesday, or Wednesday.**

Infusion scheduling & order. Cannot be given within 30 minutes of another infusion. Ferumoxytol is preferred to be the first infusion / treatment of the day if multiple treatments are given.

Preparation. Fatal and serious hypersensitivity reactions, including anaphylaxis, have occurred with ferumoxytol. Physician discretion for appropriate premedication and available rescue medications is required.

Duration. Final treatment period is prescribed to coincide with adjuvant temozolomide cycle 1 day 1.

Monitoring. Key blood tests for monitoring ferumoxytol-associated toxicity are AST, ALT, ferritin, and the transferrin (iron) saturation (TSAT). Liver and iron panel results must be reviewed by hepatologist if:

- Baseline labs (LFTs or iron panel) were within normal limits but are now outside of normal limits.
- Baseline labs (LFTs or iron panel) were elevated and remain elevated (regardless of change)

If the subject endorses right upper quadrant pain or a study physician identifies a concern for iron load, similarly, the subject will be evaluated for iron overload.

Concomitant multivitamin, iron, and/or drugs that alter iron metabolism. If a participant is being monitored for potential iron overload (as per immediately above), the subject must stop taking any iron supplementation (including a multivitamin with iron) and should have concomitant medications reviewed for interactions with iron (i.e., potential to increase iron retention) and hepatotoxicity.

Suspected iron overload evaluation. If iron overload is suspected, the following test must be performed. Order is at clinical discretion:

- Iron panel including ferritin and TSAT, liver function tests including AST and ALT, and a CBC with differential
- Hepatitis B core antibody IgM (CPT 86705), Hepatitis B surface antibody (CPT 86706), Hepatitis A antibody IgG / IgM (CPT 86708), and Hepatitis C antibody (CPT 86803)
- Liver ultrasound
- Hepatology consult for evaluation

Iron overload treatment. Treatment for suspected or known iron overload will be under the direction of an attending hepatologist. Treatment options include:

- In absence of severe anemia. Subjects will be treated with phlebotomy
- In presence of anemia. Subjects will receive chelation therapy

Subjects will also be counseled to remain off multivitamins containing iron, iron supplements, alcohol, and any hepatotoxic medications until instructed by a physician.

Infusion 1 hypersensitivity / infusion reaction. If a participant experiences signs/symptoms consistent with a hypersensitivity reaction during the first ferumoxytol infusion administered on day 0, the subject is withdrawn due to an anticipated, expected serious adverse event (SAE). This will be documented in the subject's casebook. This will not be considered a dose limiting toxicity, as the subject has not been administered therapeutic radiation, temozolomide, and ascorbate. The dose limiting toxicity window does not begin until study day 1.

Dose reductions. None. An adverse event suggesting the need for a dose reduction should be evaluated against criteria for a dose limiting toxicity.

Dose interruptions. An adverse event requiring a dose interruption meets the criteria for a dose limiting toxicity.

Dose cohorts.

Cohort 1 (starting dose level). Participants will receive a single dose of ferumoxytol during each 5 week treatment period. The first infusion is the calendar day prior to the first radiation therapy treatment. Participants in cohort 1 will receive a maximum of 3 ferumoxytol infusions.

Cohort 2. Participants will receive 2 doses of ferumoxytol during each 5 week treatment period. The second ferumoxytol infusion is administered 3 to 8 calendar days after the first infusion. Participants in cohort 2 will receive a maximum of 6 ferumoxytol infusions.

Escalation rules.

This study will evaluate up to 2 dose levels employing an escalation strategy based on observed dose-limiting toxicities.

Accrual is placed on hold after the last subject in a cohort receives at least one dose of all interventions (ferumoxytol, radiation, ascorbate, temozolomide). Dose cohorts and escalation rules are provided (Table 1). The initial cohort explores 1 administration of ferumoxytol per 5-week treatment period. An escalation to 2 administrations of ferumoxytol per 5-week treatment period if a decision rule is met. Six participants must be evaluated at the recommended phase 2 dose (RP2D).

Dose limiting toxicity window (DLT window). From study day 1 (i.e., one dose of ascorbate, temozolomide and radiation) through C2D28.

Dose limiting toxicities. Graded per CTCAE v5. A dose limiting toxicity is defined by the protocol as an unacceptable clinically significant adverse event or abnormal laboratory value assessed as unrelated to disease progression or undercurrent illness. These events were identified from the literature as well as published experiences with the treatments. The following adverse events have been defined as dose limiting toxicities for the purposes of this clinical trial:

- iron overload, defined for the purposes of this protocol as: persistent transferrin saturation of ≥ 90% and ferritin > 1000 ng/ml two weeks following ferumoxytol infusion that persists for an additional two weeks in the absence of liver abnormality (as evaluated by liver ultrasound and hepatology) and negative hepatitis serology.
- an unanticipated serious adverse event with an attribution to the study agents (pharmacological ascorbate, ferumoxytol). An unanticipated SAE is described by the FDA as one that is not consistent with the participant's known medical history, the disease under study, side effect profile of the standard of care therapy, or a comorbidity common to the population (e.g., heart disease or arthritis in the elderly). All SAEs

will be evaluated to determine if DLT criteria are met – an SAE is not necessarily a DLT if it is not reasonably associated to the investigational medical products (pharmacological ascorbate, ferumoxytol).

The DSMC will provide an independent assessment for each DLT and SAE. A robust monitoring and subsequent review of the results and data for the enrolled and treated subjects will be completed by the DSMC after every 3 treated participants.

6.1.2.2. PHARMACOLOGIC ASCORBATE

Screening dose. 15g in 250 mL of sterile water for human use administered over no less than 30 minutes. Screening dose may be repeated at PI's discretion. If given during a therapy week, the 15g dose may replace a "therapeutic" dose (i.e., a 15g dose and then two 87.5g doses in a study week)

Treatment dose. 87.5 g in 1250 mL of sterile water for human use administered 3 times per calendar week during the RT phase and 2 times per week during the interim and adjuvant phases. Schedule can be reduced to 2 times per calendar week during holidays or for inclement weather if applicable.

Route of administration. Intravenous.

Infusion rate. Do not exceed 500 mL/hour; rate can be reduced as necessary but indication for rate reduction noted in the infusion record or case history.

Initiation. Begin on radiation fraction 1.

Duration. Through completion of adjuvant temozolomide cycle 6.

Scheduling. Cannot be administered within 30 minutes of ferumoxytol or on days with iodinated contrast.

Dose reductions. None.

Dose interruptions. Hold and call if AST or ALT ≥ grade 3. Other interruptions must be approved by the investigator or designee and evaluated for a dose limiting toxicity. Rationale for the interruption must be clearly documented as well as the justification for resuming.

6.1.2.3. CONCOMITANT TEMOZOLOMIDE (I.E. DURING RADIATION)

Concomitant temozolomide will be administered as per the FDA-approved prescribing information.

Dose. 75 mg/m^2 once daily; dose may be rounded to nearest 10 mg at discretion of treating physician.

Route of administration. Oral.

Initiation. Time to day 1 of radiation therapy as per treating physicians.

Preparation. To reduce nausea and vomiting, take temozolomide on an empty stomach or at bedtime. Utilize antiemetic therapy prior to and/or following administration as necessary. Instruct participant to swallow capsules whole. Do not open or chew capsules.

Duration. Up to 49 consecutive days concomitant with radiotherapy.

Dose Reductions

No dose reduction will be made but delay or discontinuation of temozolomide administration will be decided weekly according to hematologic and non-hematologic adverse events (AEs), as specified below.

Temozolomide Dosage Modifications During Concomitant Phase		
Adverse reaction	**Interruption**	**Discontinuation**
Absolute neutrophil count	Withhold if ANC is $< 1.5 \times 10^9$/L but is $\geq 0.5 \times 10^9$/L Resume when ANC is $\geq 1.5 \times 10^9$/L.	Discontinue if ANC is $< 0.5 \times 10^9$/L
Platelet count	Withhold temozolomide if platelet count is $< 100 \times 10^9$/L but is $\geq 10 \times 10^9$/L Resume temozolomide when platelet count is $\geq 100 \times 10^9$/L.	Discontinue temozolomide if platelet count is $< 10 \times 10^9$/L
Non-hematologic adverse reaction (except alopecia, nausea, or vomiting unless on maximal antiemetic therapy)	Withhold for grade 2 or grade 3 adverse reaction at physician discretion. Resume when event resolves to $\leq$ grade 1	Discontinue for grade 4 adverse reaction

Interruptions due to hematologic AE. A complete blood count (CBC) should be performed at least twice weekly when criteria are met for temozolomide withhold. As soon as all resumption criteria in the above table have been met, the administration of temozolomide will resume at the same dose as used initially.

Interruptions due to non-hematologic AE. Assess the participant at least weekly with relevant laboratory test(s). As soon as all resumption criteria in the above table have been met, the administration of temozolomide will resume at the same dose as used initially.

Radiation during interruptions. If temozolomide is interrupted, radiotherapy may proceed. Missed doses of temozolomide will not be made up at the end of radiotherapy.

Radiation interruptions. If radiation therapy has to be temporarily interrupted for technical or medical reasons unrelated to the temozolomide administration, treatment with daily temozolomide should continue. If radiation therapy has to be permanently interrupted, treatment with concomitant temozolomide also ends.

6.1.2.4. RADIATION THERAPY

Initiation. Simultaneous with temozolomide.

Dose. 60 Gy or 61.2 Gy

Administration. 1.8 Gy / fraction or 2.0 Gy per fraction, 1 fraction per day.

Concomitant treatment. Radiation may be administered during active ascorbate infusion.

Schedule. Consecutive administration, Monday through Friday, per usual clinical schedule. First week must have a minimum of 2 consecutive fractions, beginning the calendar day after the first ferumoxytol infusion.

Duration. 30 fractions (at 2.0 Gy/fraction) or 34 fractions (1.8 Gy/fraction).

Delay. Interruptions or breaks in radiation therapy are at radiation oncologist discretion. Reason for interruption must be clearly noted in source document. Breaks of ≥ 2 consecutive business days must be reported to PIs.

Dose reductions. Not applicable.

6.1.2.5. ADJUVANT TEMOZOLOMIDE (I.E. POST-RADIATION)

Dose modifications provided in this section are based on the literature. Given the nature of GBM and poor therapeutic options, the treating physician may modify temozolomide dose as appropriate.

Dose. Orally once per day. Cycle 1 dose is 150 mg/m^2/day with a single dose escalation to 200 mg/m^2/day for cycle 2-6 if no treatment-related adverse events > grade 2 are noted. Dose may be rounded to nearest 10 mg at discretion of treating physician.

Initiation. 28 days after the last dose of radiation (± 5 business days).

Administration. Temozolomide may be taken at subject's preference provided it is a consistent time schedule. Subject can delay dosing with temozolomide per their preference, provided labs remain within 1 calendar week of taking the first dose.

Schedule. Once daily for 5 consecutive days (1 – 5) of a 28-day cycle.

Cycle. 28 days.

Delay. Obtain a complete blood count on Day 22 and then weekly until the ANC is above 1.5 x 10^9/L and the platelet count is above 100 x 10^9/L. Do not start the next cycle until the ANC and platelet count exceed these levels. **If held for ≥ 30 days, subject is removed from study.**

Dose reductions. If temozolomide is withheld, reduce the dose for the next cycle by 50 mg/m^2 per day. Permanently discontinue temozolomide in patients who are unable to tolerate a dose of 100 mg/m^2 per day.

Temozolomide Dosage Modifications During Maintenance Treatment		
Toxicity	**Interruption & dose reduction**	**Discontinuation**
Absolute neutrophil count (ANC) decreased	Withhold temozolomide if ANC < 1 x 10^9 cells/L When ANC is above 1.5 x 10^9/L, resume TEMODAR at reduced dose for the next cycle	Unable to tolerate a dose of 100 mg/m^2 per day
Platelet count decreased	Withhold temozolomide if platelet count is < 50 x 10^9 cells/L When platelet count is above 100 x 10^9 cells/L, resume temozolomide at reduced dose for the next cycle.	Unable to tolerate a dose of 100 mg/m^2 per day
Non-hematologic adverse reactions (except for alopecia, nausea, vomiting)	Withhold temozolomide at physician discretion if Grade 3 adverse reaction. When resolved to Grade 1 or less, resume temozolomide at reduced dose for the next cycle	Recurrent Grade 3 after dose reduction, or instance of Grade 4, or unable to tolerate a dose of 100 mg/m^2 per day.
Taken from U.S. FDA prescribing information for Temodar® revision 09/2023		

Duration of treatment. Subjects will be treated with adjuvant temozolomide for 6 cycles or until one of the criteria for removal is met. If a subject completes 6 cycles of adjuvant temozolomide, subject may continue temozolomide off protocol or seek other treatment options available to them.

6.2. PREPARATION/HANDLING/STORAGE/ACCOUNTABILITY

6.2.1. ACQUISITION AND ACCOUNTABILITY

6.2.1.1. TEMOZOLOMIDE (TEMODAR®)

Temozolomide is available commercially. The drug should be prescribed, dispensed, and disposed of according to institutional procedures.

6.2.1.2. PHARMACOLOGIC ASCORBATE (ASCOR®)

ASCOR® is provided by McGuff Pharmaceuticals Inc. for investigational use under the physician held IND. ASCOR® will be shipped directly to the pharmacy of record. Drug accountability and receipt logs (DAR) will be utilized to maintain receipt, dispense, and destruction. Institutional policy will be followed to destroy unused or returned drug product.

6.2.1.3. FERUMOXYTOL (FERAHEME®)

Ferumoxytol is available commercially. The drug should be prescribed, dispensed, and disposed of according to institutional procedures.

6.2.2. FORMULATION, APPEARANCE, PACKAGING, AND LABELING

6.2.2.1. TEMOZOLOMIDE (TEMODAR®)

TEMODAR (temozolomide) capsules for oral use contains either 5 mg, 20 mg, 100 mg, 140 mg, 180 mg, or 250 mg of temozolomide. The inactive ingredients are as follows:

- TEMODAR 5 mg: lactose anhydrous (132.8 mg), colloidal silicon dioxide (0.2 mg), sodium starch glycolate (7.5 mg), tartaric acid (1.5 mg), and stearic acid (3 mg).
- TEMODAR 20 mg: lactose anhydrous (182.2 mg), colloidal silicon dioxide (0.2 mg), sodium starch glycolate (11 mg), tartaric acid (2.2 mg), and stearic acid (4.4 mg).
- TEMODAR 100 mg: lactose anhydrous (175.7 mg), colloidal silicon dioxide (0.3 mg), sodium starch glycolate (15 mg), tartaric acid (3 mg), and stearic acid (6 mg).
- TEMODAR 140 mg: lactose anhydrous (246 mg), colloidal silicon dioxide (0.4 mg), sodium starch glycolate (21 mg), tartaric acid (4.2 mg), and stearic acid (8.4 mg).
- TEMODAR 180 mg: lactose anhydrous (316.3 mg), colloidal silicon dioxide (0.5 mg), sodium starch glycolate (27 mg), tartaric acid (5.4 mg), and stearic acid (10.8 mg).
- TEMODAR 250 mg: lactose anhydrous (154.3 mg), colloidal silicon dioxide (0.7 mg), sodium starch glycolate (22.5 mg), tartaric acid (9 mg), and stearic acid (13.5 mg).
- The body of the capsules is made of gelatin and is opaque white. The cap is also made of gelatin, and the colors vary based on the dosage strength. The capsule body and cap are imprinted with pharmaceutical branding ink, which contains shellac, dehydrated alcohol, isopropyl alcohol, butyl alcohol, propylene glycol, purified water, strong ammonia solution, potassium hydroxide, and ferric oxide.
- TEMODAR 5 mg: The green cap contains gelatin, titanium dioxide, iron oxide yellow, sodium lauryl sulfate, and FD&C Blue #2.
- TEMODAR 20 mg: The yellow cap contains gelatin, sodium lauryl sulfate, and iron oxide yellow.

6.2.2.2. PHARMACOLOGIC ASCORBATE (ASCOR®)

ASCOR® is supplied as a Pharmacy Bulk Package (PBP) of 25,000 mg/50 mL (500 mg/mL) which is intended for dispensing of single doses to multiple patients in a pharmacy admixture program and is restricted to the preparation of admixtures for infusion. ASCOR® is a clear, colorless to pale yellow solution.

6.2.2.3. FERUMOXYTOL (FERAHEME®)

Ferumoxytol is available in single-dose vials (17 mL) in single vial packaging or in packaging of 10 vials per carton.

6.2.3. PRODUCT STORAGE AND STABILITY

6.2.3.1. TEMOZOLOMIDE (TEMODAR®)

Store capsules at 25°C (77°F); excursions permitted to 15°C to 30°C (59°F to 86°F) [USP Controlled Room Temperature].

6.2.3.2. PHARMACOLOGIC ASCORBATE (ASCOR®)

Store in a refrigerator at 2° to 8°C (36° to 46°F). Protect from light. This product contains no preservative. Once the closure system has been penetrated, complete all dispensing from the PBP vial within 4 hours.

6.2.3.3. FERUMOXYTOL (FERAHEME®)

Store at 20° to 25°C (68° to 77°F). Excursions permitted to 15° – 30°C (59° – 86°F) [USP controlled room temperature].

6.2.4. PREPARATION

6.2.4.1. TEMOZOLOMIDE (TEMODAR®)

Preparation is not required.

6.2.4.2. PHARMACOLOGIC ASCORBATE (ASCOR®)

87.5 g (175 mL) of ascorbate should be mixed with 1250 mL sterile water for human injection, resulting in an estimated osmolarity of 795 mOsm/L (targeted range is 500 – 900 mOsm/L). The diluted ascorbate should be protected from light and refrigerated prior to use. Each prepared dose must be used within 24 hours. Discard unused portion.

6.2.4.3. FERUMOXYTOL (FERAHEME®)

510 mg (17 mL) ferumoxytol should be mixed with 50 mL of 0.9% NaCl. Once diluted, ferumoxytol should be used immediately but may be stored at room temperature (68°F to 77°F) for up to 4 hrs. The treatment team will ensure adequate access to appropriate medications to address hypersensitivity reaction (e.g., diphenhydramine, epinephrine, famotidine, methylprednisolone). Pre-medication as well as medical regimen to address potential hypersensitivity is left to the discretion of the treating physician.

6.3. MEASURES TO MINIMIZE BIAS: RANDOMIZATION AND BLINDING

This phase 1 clinical trial utilizes dose limiting toxicity as the primary endpoint. As such, bias is minimized by using independent monitors to review subject medical records and study casebooks to evaluate if adverse events were assessed appropriately and dose limiting toxicities identified as per protocol. Prior to dose escalation or determination of the recommended phase 2 dose, an independent data and safety monitoring committee (Holden Comprehensive Cancer Center, University of Iowa) will review all data to determine the appropriateness of the request.

To estimate an effect size to support a well-controlled effectiveness study, tumor measurements will be obtained.

To minimize bias, a single blind will be used in radiologic evaluations for this study. A centralized reviewer will evaluate MR scans per RANO.

Subjects, the investigator and research team, and the treating oncologists will not be blinded to the subject's therapy.

6.4. STUDY INTERVENTION COMPLIANCE

6.4.1. TEMOZOLOMIDE

This medication is administered on an outpatient basis. Compliance is assessed utilizing a pill diary reviewed weekly with licensed medical staff. Prescription and dispense records will also be reviewed. To be considered evaluable, a subject must have completed 80% of prescribed temozolomide for radiation, interim, and cycles 1 & 2.

6.4.2. PHARMACOLOGIC ASCORBATE

Pharmacologic ascorbate infusions are administered by licensed medical staff. For this reason, adherence to protocol will be assessed through source document verification in the electronic health records for prescription, dispense, and administration. To be considered evaluable, a subject must have completed 80% of prescribed ascorbate for radiation, interim, and cycles 1 & 2.

6.4.3. FERUMOXYTOL

Ferumoxytol infusions are administered by licensed medical staff. For this reason, adherence to protocol will be assessed through source document verification in the electronic health records for prescription, dispense, and administration.

To be considered evaluable for the assigned cohort, a participant must have completed the assigned ferumoxytol infusions as prescribed per cohort unless a stopping rule was met.

6.5. CONCOMITANT THERAPY

For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician. Due to the first-in-human phase 1 design, concomitant medications (medications taken, not simply prescribed or purchased) should be reconciled no less than weekly.

- **Concurrent infusions.** Allow at least **30 minutes between the administration of ferumoxytol and administration of other medications** that could potentially cause serious hypersensitivity reactions and/or hypotension, such as chemotherapeutic agents or monoclonal antibodies.[35]
- **Glucometers**. Pharmacologic ascorbate creates false results for finger-stick glucose readings.[64,66] This effect is dose dependent but is known to last up to 6 hours post-infusion. Serum/plasma tests are not affected.

Individuals who develop a need to self-check blood glucose with fingerstick glucometers should be removed from study.

- **FDG PET scans**. Imaging using a glucose-based PET scan is susceptible to error within 24 hours of an ascorbate infusion due to the falsely elevated finger stick glucose readings.[67,68] Discuss FDG PET imaging with allied healthcare staff and schedule accordingly.
- **Oral iron preparations are prohibited**.[35]
- **Imaging contrast**. Imaging contrast for CT or PET scans should not be administered on the same day as an ascorbate infusion. If administered within the same day, monitor liver function tests and contact the medical monitors for the IND. Contrast for MR imaging, including gadolinium, is acceptable.
- **CYP450 3A4 interactions**. Review drugs for the potential for interactions. Medically monitor subjects receiving a potentially interactive drug.
- **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.
- **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.
- **Tumor treating fields (TTF).** Due to the phase 1 nature of this clinical trial, tumor treating fields are not allowed during active therapy and the DLT window (from ferumoxytol infusion 1 through the end of adjuvant cycle 2). Participants may utilize TTF at the discretion of their study physician after completing cycle 2 (i.e. end of dose limiting toxicity window).
- **Investigational drugs and/or devices.** Investigational use of drugs and/or devices in a clinical trial or human subjects study are prohibited from consent until study completion. Initiation of a clinical trial prior to the 30 day follow-up should be discussed with the sponsor-investigator. Prohibition is regardless of indication for study drug and/or investigating off-labelled use. Imaging agents and/or radiologic studies may be performed with prior approval of the sponsor-investigator.

6.5.1. RESCUE MEDICINE

Fatal and serious hypersensitivity reactions including anaphylaxis have occurred with ferumoxytol. To minimize risk, ferumoxytol must only be administered under the care of appropriately trained personnel and with therapies immediately available for the treatment of anaphylaxis and other hypersensitivity reactions.

7. STUDY INTERVENTION DISCONTINUATION AND PARTICIPANT DISCONTINUATION OR WITHDRAWAL

7.1. DISCONTINUATION OF STUDY INTERVENTION

A subject's discontinuation of the study intervention (i.e., declining further study treatment) does not define the subject as being off study (protocol section 7.2). **If the subject consents to continue participating in study procedures, all study procedures should continue even in the absence of study treatment.** Subjects who decline further treatment should be reviewed for a dose-limiting toxicity by the investigator and DSMC.

- **Serious adverse event attributable to investigational product.** If the subject experiences a serious adverse event and it is reasonably attributed to the investigational product, the subject will be withdrawn from treatment and followed as per protocol. The investigator must notify the DSMC within 1 business day and file with the IRB of record.

- **Subjects may decline further study intervention**. If a subject who has tolerated the first dose of ferumoxytol declines further study intervention, the reason for declining treatment should be documented. Specifically, if the discontinuation is due to an AE. This is required for federal reporting.
- **Unforeseen medical event.** If a subject undergoes a medical event that compromises the subject's safety or study's endpoints, the subject may be withdrawn from treatment. The sponsor-investigator or investigator may make this determination. If treatment is ended for this reason, the subject should be followed for a minimum of 30 calendar days from the last injection with investigational product.
- **Pregnancy**. If a subject declares pregnancy, the subject will be withdrawn. The sponsor will be notified within 1 business day of the event through the serious adverse event reporting mechanism. The IRB of record must be notified.
- **Subject non-compliance.** If a subject does not comply with the required adverse event assessment (i.e., hematologic and serum chemistry assessments, follow-up assessments), study treatment may be discontinued to reduce risk to the subject.

7.2. PARTICIPANT DISCONTINUATION/WITHDRAWAL FROM THE STUDY

Subject participation may discontinued (i.e., withdrawn from study) in the following circumstances.

- **Subjects who decline to receive any study treatment after consenting** to the clinical trial are withdrawn from study and are not followed for this clinical trial.
- **Subjects who experience a reaction** to the first dose of ferumoxytol that, in the opinion of the investigator, increases risk to subject will be considered a screen fail and be withdrawn from study. They will not be followed for this trial.
- **Investigator non-compliance**. If an investigator is considered to be non-compliant with the study protocol or federal, state, local, or institutional requirements, the principle investigators may end the study and withdraw subjects from trial.
- **Subject refusal to continue.** Subjects who decline further study follow-up (i.e. no longer wish to undergo study assessments) are withdrawn from the study. If the participant withdraws full permission (i.e., revokes HIPAA authorization), the participant will not be followed further, but all data obtained until the date of HIPAA revocation will be used for the purposes of this study. Subject's intent must be well documented in the case book.
- **Termination of study.** If the study is terminated for any reason, the subject will be followed for a minimum of 30 calendar days for safety assessment, including hematologic and serum chemistries as prescribed per protocol.

7.3. LOST TO FOLLOW-UP

A subject is considered lost to follow-up for the purposes of this trial if one of the following criteria are met:

- Cancels (without explanation) or fails to appear for treatments
- Does not return follow-up phone calls from research staff (three attempts, minimum)
- Written notice (certified mail) to subject returns 'address unknown.'

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- **Safety assessments.** The site will attempt to contact the participant and reschedule missed laboratory assessments or physical exams within 3 business days. Research staff or the treating study physician should counsel the participant on the importance of safety assessments. **Site research staff will ask the subject if they are willing to continue in the study**. If subject has seen local practitioner *in lieu* of UIHC oncologist, obtain release of information and obtain local provider notes. **A participant's failure to return to the study**

site for assessments is not considered a deviation if local assessments can be obtained as per study calendar.

- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up. This should be reported to the IRB of record per institutional policies.

8. STUDY ASSESSMENTS AND PROCEDURES

8.1. EFFICACY ASSESSMENTS

8.1.1. SCREENING PROCEDURES

8.1.1.1. SCREENING – STEP 1

The following must be completed prior to the first ferumoxytol infusion as well as the 15 gram pharmacologic ascorbate test dose. At minimum, eligibility must be confirmed by a study physician. Screening tests may be completed as necessary to confirm eligibility. **Due to the turn around time from surgery to treatment day 0 (6 weeks) a screening assessment window is not employed**.

- **Informed consent**. Consent must be obtained as per the IRB of record specifications. Legally authorized representatives will not be utilized. Consent must be obtained prior to testing for G6PD.
- **Pathology.** Confirm pathologic diagnosis to be glioblastoma or diffuse midline glioma. Pathologic analysis may be historical (prior to consent).
- **Physical exam.** A physical exam must be performed to assess ECOG status, medical fitness for therapy (temozolomide and radiation), co-morbid diseases, and uncontrolled intercurrent illnesses. Medication list should be reviewed for prohibited medications at this visit. Physical exam may be completed by medical oncology or radiation oncology and may be historical (i.e. prior to consent); if historical, a research team member must review concomitant medication(s) and medical history for exclusion criteria. A single exam may be used for both screening and baseline requirements.
- **G6PD**. Draw as soon as possible after consent as this is a mail out test, delaying results for up to 1 week. If the test returns less than the lower limit of normal, at PI discretion the ascorbate infusions may be held for up to 10 days for retesting.
- **Platelet and creatinine**. Values from labs obtained post-surgery or post-biopsy may be used. Labs may be repeated to meet eligibility criteria.
- **Pregnancy test**. An individual of child-bearing potential per institutional policy must have a negative pregnancy test (urine or blood) prior to initiating therapy.

8.1.1.2. SCREENING – STEP 2

The following assessments should be completed only after step 1 eligibility has been confirmed. Prior to initiating either infusion, baseline labs must be drawn and resulted (PT/INR, uric acid, CBC w/diff, comprehensive metabolic panel, and iron panel with ferritin). If the first therapeutic dose of ferumoxytol is to be administered the same day the order of administration is 15g test dose first, then confirmation of eligibility, and then the ferumoxytol (at least 30 minutes after the 15g test dose).

- **15 gram ascorbate test dose**. A 15 gram infusion of pharmacologic ascorbate must be tolerated by the participant. Tolerance is determined qualitatively by the investigator, but infusion reactions include hypotension, bronchospasm, hypoxia, and syncope.

  Note – assessments required prior to the 15 g test dose. Before the 15g ascorbate dose, the following must be completed to ensure a true baseline. These data and samples will be destroyed if the subject is a screen fail or has a reaction to the ferumoxytol infusion on day 0.
  - **T2* MR imaging.** T2* MRI may be done at any time post-consent but prior to 15g test dose.
  - **Baseline ascorbate levels**. Draw in a 4 mL green-top tube (sodium heparin).
  - **Patient reported outcome measures**
  - **Optional research blood**. If participant has opted in, draw 1 4cc purple top EDTA tube for immunology samples

If a participant does not tolerate the 15 gram ascorbate test dose they are considered a screen fail due to an adverse event.

8.1.2. BASELINE ASSESSMENTS

The following should be completed within 21 days of day 0. Assessments already completed for standard of care can be utilized in compliance with institutional procedures and HIPAA compliance. All assessments are completed using CTCAE v5. Laboratory assessments should be completed and resulted prior to the 15g test dose and first ferumoxytol infusion.

Although adverse events are being examined for an efficacy endpoint to determine the combined therapy's effect on symptoms of treatment and/or disease, they are foundation of safety assessment for investigational agents. **For this reason, adverse event collection is described under section 8.2**.

1. Physical exam, including vital signs and neurologic assessment for neuro-oncology (NANO) scale. This can be historical exam provided it is post-operative / post-biopsy.
2. Patient Reported Outcome Measures (PROMs).
3. PT & INR
4. Uric acid
5. Iron panel & ferritin
6. CBC with differential
7. Comprehensive metabolic panel (CMP)

8.1.3. ACTIVE THERAPY

The following will be performed during active therapy and/or data will be mined.

1. **Ascorbate levels**. Draw 1 NaHep tube at the following time points as soon as is reasonably possible after completing the ascorbate infusion:
   - the first 87.5 infusion
   - at each cohort-dependent MR imaging timepoint during RT (Cohort 1: at last T2* day after FMX infusion 2; Cohort 2: at last T2* the day after FMX infusion 2)
   - the last ascorbate infusion during radiation therapy
   - for Cohort 2, at the last T2* the day after FMX infusion 4 (regardless of association with radiation therapy)for Cohort 2, at the last T2* the day after FMX infusion 6
   - adjuvant cycle 1 (days 22-28)

   Note. Pre-infusion levels are not drawn.
2. **Immunology samples** / exploratory blood (optional). Draw 1 4-cc purple top EDTA tube at the following timepoints:
   - Pre-temozolomide at Cycle 1
   - 7 days post-adjuvant cycle 1 (± 1 day)
3. **Research iron levels**. Do not process clinically as these are not for clinical assessment. Draw prior to any treatment (radiation, ascorbate, or temozolomide) but can be drawn after imaging. Note: If a clinical iron level is assessed within the same time (± 3 business days, or with PI approval), the research iron level can be omitted.
   - at each cohort-dependent MR imaging timepoint during RT (Cohort 1: at last T2* day after FMX infusion 2; Cohort 2: at last T2* the day after FMX infusion 2 and infusion 4)
   - at each cohort-dependent MR imaging timepoint during adjuvant temozolomide (Cohort 1: at last T2* day after FMX infusion 3; Cohort 2: at last T2* the day after FMX infusion 6)
4. **Patient Reported Outcome Measures (PROMs).** Administer patient completed questionnaire weekly during the radiation phase at the weekly physician visit during treatment. Administer on the day of medical oncology visit for each adjuvant temozolomide cycle and the 30-day follow-up.

8.1.3.1. QSM/T2* IMAGING

MR imaging will be performed on a 3T Siemens TIM Trio scanner. Each imaging session will acquire a 3D multi-echo gradient-echo pulse sequence (TE = 7, 14, 21, 28, 35, 42, 49, and 56 ms; TR = 80 ms; matrix size = 256x200x104; FOV = 220 × 220 x 156 mm; slice thickness = 1.5mm, flip angle = 17°, bandwidth=300Hz/pixel) for T2* and QSM mapping as well as 3D T1-weighted SPACE variable flip angle fast spin-echo sequence, and FLAIR sequence.

QSM/T2* MRI is dependent on cohort assignment (see Schemas and Schedule of Activities).

Cohort 1 will have T2*/MR imaging collected at the following timepoints; sequence details are provided in schema:

- **Baseline:** Prior to 15 g ascorbate test dose (target RT simulation if possible)
- **Study day 1**: Prior to any treatment and then 2 to 4 hours post pharmacological ascorbate infusion.
- **Boost imaging**: Boost imaging is independent of T2* imaging for this cohort; if due to delays, the timing aligns, T2* may be done concurrently with boost imaging (it is not prohibited).
- **Calendar day after ferumoxytol infusion 2:** This imaging is independent of boost imaging for treatment planning. Complete the day after the second ferumoxytol infusion. Obtain T2* prior to any treatment and then 2 to 4 hours post pharmacological ascorbate infusion (note: the iron panel should be drawn with the second T2* imaging session).
- **Calendar day after ferumoxytol infusion 3**: Complete the calendar day after the third ferumoxytol infusion. Obtain T2* prior to pharmacological ascorbate infusion and then 2 to 4 hours post

pharmacological ascorbate infusion (note: cycle 1's iron panel should be drawn with the second T2* imaging session).

Cohort 2 will have T2*/MR imaging collected at the following timepoints:

- **Baseline:** Prior to 15 g ascorbate test dose (target RT simulation if possible)
- **Calendar day after ferumoxytol infusion 2:** Prior to any treatment and then 2 to 4 hours post pharmacological ascorbate infusion for a total of 2 T2* imaging sessions.
- **Boost imaging**: Boost imaging is independent of T2* imaging for this cohort; if due to delays, the timing aligns, T2* may be done concurrently with boost imaging (it is not prohibited).
- **Calendar day after ferumoxytol infusion 4:** Obtain T2* prior to any treatment that day (ascorbate and, if appropriate, RT) and then 2 to 4 hours post pharmacological ascorbate infusion for a total of 2 T2* imaging sessions. (note: the iron panel should be drawn with the second T2* imaging session).
- **Calendar day after ferumoxytol infusion 6**: Complete the day after the sixth ferumoxytol infusion. Obtain T2* prior to pharmacological ascorbate infusion and then 2 to 4 hours post pharmacological ascorbate infusion for a total of 2 T2* imaging sessions (note: the iron panel should be drawn with the second T2* imaging session).

8.1.3.2. DATA MINING

1. Radiation dose to tumor.
2. Elapsed days to complete prescribed radiation.
3. Number of radiation breaks.
4. Number of break days. If a break is on a Friday, the following weekend and/or holiday will be included in the break until radiation resumes.
5. Expansion margins (GTV to CTV, CTV to PTV).

8.1.4. ACTIVE FOLLOW-UP

Protocol prescribed active follow-up is 2 years from the final therapeutic infusion. The dose limiting toxicity window is through adjuvant cycle 2, day 28 (including the diagnostic MRI). The following data will be collected during the follow-up phase of the study as noted.

- **Protocol-recommended imaging.** Standard of care is imaging should be performed per NCCN guidelines. Any CT or MR imaging obtained for routine clinical follow-up will be used to assess efficacy of treatment. Appropriate authorization should be obtained. Digital images should be obtained.
- **Protocol-dictated diagnostic MRI.** Protocol requires imaging once between 3 to 4 months after the last fraction of radiation therapy. It is strongly recommended, but not required, that protocol imaging be completed at UIOWA or another QIBA (or NCI) accredited imaging center.

8.1.5. LONG TERM FOLLOW-UP

After completing active follow-up, data will be collected twice per year to assess progression (as appropriate) and overall survival status. Given appropriate institutional approval and authorizations, this can be done through chart review, phone call to the participant, or contacting a local provider. Subject will be followed until the study is completed, the subject succumbs to disease, or the subject withdraws consent for further study follow-up.

8.2. SAFETY AND OTHER ASSESSMENTS

8.2.1. HISTORICAL INFORMATION

The following data should be mined, if available, from the subject's existing medical records. If information is available from outside medical facilities, the investigator and research staff are requested to obtain appropriate authorization from the subject to obtain copies of these data. **Only the most recent imaging should be obtained for each category** (e.g., MRI, CT, PET/CT).

- **Significant medical conditions**, defined as those conditions that could contribute to underlying health conditions or treatment-emergent adverse events. Examples include, but are not limited to, hypertension, prior myocardial infarction, seizures, and sepsis.
- **Metabolic and hematologic baselines**. Baseline metabolic and hematologic conditions will be obtained from screening labs and/or any hematology or comprehensive metabolic panels. These conditions will be marked as "active," in the electronic database (EDC) and graded per CTCAE v.5. If subjects have a long standing and established hematologic (e.g., anemia) or metabolic (e.g., hyperglycemia) condition that is not captured on screening or baseline lab assessments, this will be marked as "past," or "not current," in the EDC and severity of the prior event can be provided in a comment in the EDC.

8.2.2. BASELINE ASSESSMENTS

The following should be completed within 21 days of study day 0. Assessments already completed for standard of care can be utilized per institutional procedures and HIPAA compliance. Assessments are completed using CTCAE v5.

1. **ECG** (≤ 21 days study day 0)
2. **Vitals**. Temperature, heart rate, blood pressure. Document in electronic health record.
3. **Solicited adverse events.** Quantify nausea, vomiting, diarrhea, fatigue, skin condition (screen against rash and itching) and pain (note location of pain, if any). Note etiology, if available.
4. **Unsolicited adverse events.** Ask participant if there are any other symptoms they are experiencing through general query. If participant endorses a symptom, note etiology, if available.
5. **Concomitant medications.** Reconcile with indication, frequency, and active use in the medical record.

8.2.3. ACTIVE THERAPY

The following data will be collected to assess safety and further determine the adverse event profile of this combination therapy. The **radiation (RT) phase** is defined as the period of time when radiation is administered in addition to temozolomide, ascorbate, and ferumoxytol. The **interim phase** is defined as the roughly 4-week period from last dose of radiation to the start of adjuvant temozolomide. The **adjuvant (ADJ) phase** is defined as six 28-day cycles of temozolomide.

1. **Labs during radiation.** Beginning week 1, draw a CBC w/diff weekly.
2. **Labs during adjuvant phase.** Beginning on cycle 1 day 1, draw a CBC w/diff on cycle day 1, and the end of cycle 6.
3. **Liver function tests.** Draw ≤ 72 hours of each ferumoxytol infusion. Mandatory labs are AST, ALT, ALP, and TBIL. If labs hemolyze, they must be redrawn. Test results must be reviewed by a study physician prior to ferumoxytol infusion.
4. **Clinical ferritin and iron panel.** Draw concurrently with LFTs before first ferumoxytol infusion. Draw 2 weeks (± 3 business days) after the last infusion in the ferumoxytol period
5. **Adjuvant CBC w/differential.** During adjuvant cycles, a CBC w/differential should be drawn on cycle day 22 (where day 1 of temozolomide defines cycle day 1).
6. **Physician's visit during radiation**. Subjects should see be seen weekly by a treating radiation oncologist during the radiation phase. The NANO scale should be completed at each scheduled weekly visit.
7. **Physical exams during adjuvant phase**. Beginning with C1 D1 of adjuvant therapy, the prescribing physician for the temozolomide should meet with the subject and review the subject's care ≤ 7 calendar

days before cycle day 1 (where day 1 is the day the subject begins temozolomide). The NANO scale should be completed at this exam.

8. **Performance status.** An ECOG or KPS performance status should be noted weekly during radiation and concurrent with the physical exams during the adjuvant phase.
9. **Solicited adverse events**. Query nausea, vomiting, diarrhea, fatigue, skin condition (e.g. rash, itching) and pain (note location of pain, if any) against CTCAE v5. Note etiology, if available. Preferably, an assessment should be done on RT *fini* day. AEs should be solicited weekly during radiation and cycle day 1 during the adjuvant phase.
10. **Pill diary**. Subjects will complete a pill diary to document temozolomide administration. Frequency of collection is at the discretion of the treatment team, but it is strongly recommended the pill diary be reviewed week 1 to verify subject is taking temozolomide during the weekend(s).
11. **Infusion assessments**. Obtain blood pressure pre- and post-ascorbate infusion. Pre-infusion blood pressure can be obtained any time prior to infusion; post-infusion blood pressure must be ≤ 15min end of ascorbate infusion.

8.2.4. ACTIVE FOLLOW-UP

Protocol prescribed active follow-up is 2 years from final ascorbate infusion. The dose limiting toxicity window is through adjuvant cycle 2, day 28. The following data will be collected during the follow-up phase of the study as noted.

8.2.4.1. 1 MONTH FOLLOW-UP

The first follow-up visit is scheduled approximately 26 to 35 calendar days after the last pharmacologic ascorbate infusion. It may be scheduled to overlap with routine clinical appointments as appropriate.

- **Physical and performance status.** Subjects should have a medical visit to review adverse events and treatment effect for review of ascorbate/ferumoxytol adverse events. Clinical follow-up should then return to standard of care at the discretion of the treating physicians.
- **Solicited adverse events**. Query nausea, vomiting, diarrhea, fatigue, skin condition (e.g. rash, itching) and pain (note location of pain, if any) against CTCAE v5. Note etiology, if available. This is the last protocol dictated solicited adverse event timepoint.
- **CBC with differential**. Draw before assigned chemotherapy, if possible. Note last chemotherapy dose.
- **Comprehensive Metabolic Panel (CMP).** Draw before assigned chemotherapy, if possible. Note last chemotherapy dose.
- **Ferritin and iron panel.**

8.2.4.2. SUBSEQUENT FOLLOW-UP

Protocol prescribed active follow-up is 2 years from the final therapeutic pharmacological ascorbate infusion to identify potential long term / chronic radiation-related adverse events. Notes and available data should be evaluated by a treating physician or designee for radiation therapy associated sequelae. This is key to determine what impact, if any, the combined regimen has on long-term effects of radiation. The following data will be collected during the follow-up phase of the study as noted.

- **Physical and performance status.** Follow-up per standard of care at the discretion of the treating physicians.
- **Ferritin and iron panel.** If iron and/or ferritin was elevated at 1 month follow-up, physician discretion to determine if additional tests should be ordered during follow-up period.
- **MRI / CT.** Imaging ordered for clinical care should be evaluated using RANO criteria for disease progression.
- **Adverse events.** Due to the nature of radiation toxicities, subjects should be followed for long-term radiation adverse events to determine the severity and frequency for comparison against published controls.
- **Tissue sample** (optional). If participant undergoes re-resection, tissue specimen obtained at investigator discretion and per subject consent.

- **Exam notes**. If subject no longer returns to UIHC for routine oncologic care, copies of physical exams and physician notes for routine follow-up should be obtained. Enter into local EHR as per standard institutional practice. Appropriate authorization should be obtained for access to information.
- **Emergency room / admission documents**. If subject is seen at an emergency room or admitted for any reason within 12 months of final pharmacological ascorbate infusion, at minimum discharge summary should be obtained. The preferred information is intake summary, initial history and physical, and discharge summary. Supporting laboratory, radiology, and surgery notes are also required.

8.2.5. LONG-TERM FOLLOW-UP

Safety data are not collected beyond 2 years post-therapy unless there is an untoward event that cannot be otherwise explained (disease, medical history, chemotherapy, other treatments).

8.3. ADVERSE EVENTS AND SERIOUS ADVERSE EVENTS

8.3.1. DEFINITION OF ADVERSE EVENTS (AE)

This study follows the United States Food & Drug Administration's definition of adverse event (AE). Specifically, the term 'adverse event' means any untoward medical occurrence associated with the use of an intervention in humans, **whether or not considered intervention-related** (21 CFR§312.32(a)).

8.3.2. DEFINITION OF SERIOUS ADVERSE EVENTS (SAE)

An adverse event (AE) or suspected adverse reaction is considered serious if, in the view of either the investigator or sponsor, it results in any of the following outcomes:

- death,
- a life-threatening adverse event,
- inpatient hospitalization or prolongation of existing hospitalization,
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions,
- a congenital anomaly/birth defect.

An important medical event is one that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgement, they may jeopardize the participant and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

8.3.3. CLASSIFICATION OF AN ADVERSE EVENT

8.3.3.1. SEVERITY OF EVENT

All adverse events, regardless of attribution, will be graded for severity using the Common Terminology Criteria for Adverse Events (CTCAE) version 5. Investigators will be provided sponsor-developed quick-start guides and instructions for adverse events of special significance.

8.3.3.2. RELATIONSHIP TO STUDY INTERVENTION

Due to the small sample size and oncologic nature of this study leading to an anticipated toxicity profile, adverse events that are mild or moderate (i.e., CTCAE grade 1 or 2) will not be evaluated for attribution to the investigational medical product. [69]

Attribution to the investigational medical therapy is required for:

- Grade 3 or 4 events
- Serious adverse events

Initial assessment may be made by any research team member with appropriate delegation. Final assessment must be determined by the site's investigator. Temporal relationship must be considered as well as pharmaceutic properties. The following definitions are provided as guidance:

- **Definitely Related** – There is clear evidence to suggest a causal relationship, and other possible contributing factors can be ruled out. The clinical event, including an abnormal laboratory test result, occurs in a plausible time relationship to study intervention administration and cannot be explained by concurrent disease or other drugs or chemicals. The response to withdrawal or reduction of the study intervention should be clinically plausible. The event must be pharmacologically or phenomenologically definitive, with use of a satisfactory rechallenge procedure if necessary.
- **Probably Related** – There is evidence to suggest a causal relationship, and the influence of other factors is unlikely. The clinical event, including an abnormal laboratory test result, occurs within a reasonable time after administration of the study intervention, is unlikely to be attributed to concurrent disease or other drugs or chemicals, and follows a clinically reasonable response on withdrawal or reduction. Rechallenge information is not required to fulfill this definition.
- **Potentially Related** – There is some evidence to suggest a causal relationship (e.g., the event occurred within a reasonable time after administration of the trial medication). However, other factors may have contributed to the event (e.g., the participant's clinical condition, other concomitant events). Although an AE may rate only as "possibly related" soon after discovery, it can be flagged as requiring more information and later be upgraded to "probably related" or "definitely related", as appropriate.
- **Unlikely to be related** – A clinical event, including an abnormal laboratory test result, whose temporal relationship to study intervention administration makes a causal relationship improbable (e.g., the event did not occur within a reasonable time after administration of the study intervention) and in which other drugs or chemicals or underlying disease provides plausible explanations (e.g., the participant's clinical condition, other concomitant treatments).
- **Not Related** – The AE is completely independent of study intervention administration, and/or evidence exists that the event is definitely related to another etiology. There must be an alternative, definitive etiology documented by the clinician.

For purposes of FDA reporting, the sponsor-investigator is responsible for final determination of relationship to study intervention.

8.3.3.3. EXPECTEDNESS

Expected adverse reactions (i.e., adverse events associated with the use of the drug) are AEs that are known to occur for the study intervention being studied and should be collected in a standard, systematic format using a grading scale based on functional assessment or magnitude of reaction. Identify the source of the reference safety information used to determine the expectedness of the AE (e.g., IB, approved labeling). Expectedness is assessed based on the awareness of AEs previously observed, not on the basis of what might be anticipated from the properties of the study intervention.

An AE or suspected adverse reaction is considered "unexpected" if it is not listed in the IB, package insert, or device labeling or is not listed at the specificity or severity that has been observed; or, if an IB is not required or available,

is not consistent with the risk information described in the protocol, as amended. For example, under this definition, hepatic necrosis would be unexpected (by virtue of greater severity) if the IB or package insert referred only to elevated hepatic enzymes or hepatitis. Similarly, cerebral thromboembolism and cerebral vasculitis would be unexpected (by virtue of greater specificity) if the IB or package insert listed only cerebral vascular accidents. "Unexpected," as used in this definition, also refers to AEs or suspected adverse reactions that are mentioned in the IB, package insert, or device labeling as occurring with a class of drugs (or other medical products) or as anticipated from the pharmacological properties or other characteristics of the study intervention, but are not specifically mentioned as occurring with the particular study intervention under investigation.

The IND sponsor will be responsible for determining whether an adverse event (AE) is expected or unexpected. An AE will be considered unexpected if the nature, severity, or frequency of the event is not consistent with the risk information previously described for the study intervention.

8.3.3.4. ANTICIPATED

Anticipated adverse events are AEs that are known to occur for the study population, condition under study, or standard treatment for the condition under study.[69] These events are established through peer-reviewed literature, U.S. prescribing information for standard therapies, and conventional medical texts. Anticipated events well established for the condition, its therapy, or the patient population should be described in the investigational plan and the expected frequency of the events provided with appropriate citations.

Shifts in frequency, severity, or onset of anticipated events must be considered as an adverse reaction related to the investigational medical product. Such changes in anticipated events should be identified through established statistical methods as available. The sponsor is responsible for identifying these shifts through data and safety review. Once a shift has been identified it should be reported to FDA as per 21 CFR 312.32 with investigators appropriate informed.

8.3.4. TIME PERIOD AND FREQUENCY FOR EVENT ASSESSMENT AND FOLLOW-UP

The occurrence of an adverse event (AE) or serious adverse event (SAE) may come to the attention of study personnel during study visits and interviews of a study participant presenting for medical care, or upon review by a study monitor.

All AEs, including local and systemic reactions not meeting the criteria for SAEs, will be documented in the participant's casebook and then entered into the appropriate case report form (CRF). Information to be collected includes event description (i.e., CTCAE v5 term), date of onset, clinician's assessment of severity (i.e., CTCAE grade), association to the investigational product(s), and date of resolution (or new baseline, resolved with sequelae). All AEs occurring while on study must be documented appropriately regardless of relationship. All AEs will be followed to adequate resolution.

Any medical condition that is present at the time that the participant is screened will be considered as baseline and not reported as an AE. However, if the study participant's condition deteriorates at any time during the study, it will be recorded as an AE. Similarly, if the etiology appears to change (i.e., a rash that disappears and then returns), this may be captured as an adverse event at the discretion of the research team / study investigator.

This study will utilize toxicity over time reporting. This requires changes in AE severity to be documented with appropriate start and stop dates, enabling an assessment of how many days of the adverse event the subject experienced. **The use of the term 'intermittent' is not allowed** as a modifier. Any adverse event described by the subject should have start & stop dates listed as completely as possible capturing onset and duration of each episode.

As per Federal Code and ICH E6, the principal investigators have overall authority for the recording, assessment, and attribution of each adverse event. The principal investigators may delegate identification, recording, and initial assessment to those individuals believed to have appropriate training and experience. The principal investigators must provide final approval on all adverse event data. All adverse events will be followed through 21 days after the

last exposure of the investigational product. All serious adverse events will be followed until resolution (i.e., return to baseline or establishment of a new baseline for that subject).

- **Solicited AEs**. Adverse events for nausea, fatigue, and pain will be queried by staff as described in section 8.2. Adverse events will also be collected directly from the participant using the NCI's PRO-CTCAE system.
- **Unsolicited AEs.** Other adverse events not directly queried will be mined from clinical notes during the time frames specified in protocol section 8.2. Additionally, these adverse events will be collected using the question, "Have you noticed anything difference since you received the injection of the investigational drug?"

8.3.5. ADVERSE EVENT REPORTING

Routine adverse events will be reported to the sponsor and the Data and Safety Monitoring Committee (DSMC) through routine electronic data capture utilizing the OnCore clinical trials management system.

8.3.5.1. DETERMINATION OF RELATIONSHIP TO INVESTIGATIONAL PRODUCT

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 sponsor-investigator (PI). The sponsor-investigator in collaboration with study investigators will determine the final relationship of the event to the investigational medical therapy (ferumoxytol and pharmacologic ascorbate):

- Grade 1 and 2 events do not require attributions assigned.
- Grade 3 and higher adverse events require attribution assigned to ferumoxytol, pharmacologic ascorbate, and the combined regimen.
- All serious adverse events (SAEs) require attribution to ferumoxytol, pharmacologic ascorbate, and the combined regimen.

Toxicity will be graded according to NCI's Common Toxicity Criteria (CTCAE v5). The sponsor-investigator will have final responsibility for determining the attribution of toxicity as it is related to the investigational product when reported to the sponsor. The sponsor will have final responsibility for assessing relationship to the investigational product when considering reporting requirements under 21 CFR§312.32

8.3.6. SERIOUS ADVERSE EVENT REPORTING

Any serious adverse events attributed to pharmacologic ascorbate, ferumoxytol and/or the combined regimen will require a notification to the DSMC within 1 business day. These expedited adverse event reports (requirements listed below) will be reviewed by the DSMC. The investigator will continue to follow or obtain documentation until the resolution of such an event until it is resolved (with or without sequelae).

All serious adverse events (SAEs) will be followed until satisfactory resolution or until the site investigator deems the event to be chronic or the participant is stable. Other supporting documentation of the event may be requested by the sponsor-investigator and should be provided as soon as possible.

8.3.6.1. SPONSOR NOTIFICATION

Not applicable; sponsor-investigator

8.3.6.2. FDA NOTIFICATION

The sponsor-investigator is responsible for notifying the Food and Drug Administration (FDA) of any unexpected fatal or life-threatening suspected adverse reaction as soon as possible, but in no case later than 7 calendar days after the sponsor-investigator's initial receipt of the information. In addition, the sponsor-investigator must notify FDA and all participating investigators in an Investigational New Drug (IND) safety report of potential serious risks, from

clinical trials or any other source, as soon as possible, but in no later than 15 calendar days after the sponsor-investigator determines that the information qualifies for reporting.

8.3.7. REPORTING EVENTS TO PARTICIPANTS

The sponsor-investigator will review all reported serious adverse events and determine the appropriateness of changes to the informed consent document. The sponsor-investigator will notify all other investigators if re-consenting subjects is mandatory for all subjects or only for those currently undergoing investigational procedures. If the sponsor-investigator does not believe re-consenting is mandatory, the investigator may require re-consent for those all subjects or just those subjects undergoing investigational procedures.

The institutional review board of record will be made aware of serious adverse events consistent with institutional policy. The sponsor-investigator will defer to the IRB if requested to change the risk section of the informed consent document. Similarly, the sponsor-investigator will also defer to the IRB if it is determined re-consent should be mandatory.

8.3.8. EVENTS OF SPECIAL INTEREST

Anticipated constitutional adverse events related to the investigational products include:

- Secondary malignancies
- Transient elevated blood pressure (i.e., an increase of diastolic BP > 15 mmHg, and/or an increase of >30 mmHg systolic, that resolves in ≤ 30 minutes)
- Dry mouth
- Seizure
- Liver injury
- Urolithiasis
- Gout
- Erythema
- Allergic reaction

Adverse events of special interest should be evaluated as described in protocol section 8.2.

8.3.9. REPORTING OF PREGNANCY

If a study participant is discovered to be pregnant after administration of the investigational products, the sponsor will be notified within 1 business day. The sponsor-investigator will provide dosimetric estimates to share with the participant's physician(s). The institutional review board of record will be notified. The participant and fetus will be followed through delivery. If a separate consent is required from the IRB of record, this consent will be requested from the participant.

8.4. UNANTICIPATED PROBLEMS

8.4.1. DEFINITION OF UNANTICIPATED PROBLEMS (UP)

The phrase "unanticipated problems involving risks to subjects or others" is found but not defined in the HHS regulations at 45 CFR part 46. OHRP considers unanticipated problems, in general, to include any incident, experience, or outcome that meets **all** of the following criteria:

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

OHRP recognizes that it may be difficult to determine whether a particular incident, experience, or outcome is unexpected and whether it is related or possibly related to participation in the research. OHRP notes that an incident, experience, or outcome that meets the three criteria above generally will warrant consideration of substantive changes in the research protocol or informed consent process/document or other corrective actions in order to protect the safety, welfare, or rights of subjects or others

Only a small subset of adverse events occurring in human subjects participating in research will meet these three criteria for an unanticipated problem.

Furthermore, there are other types of incidents, experiences, and outcomes that occur during the conduct of human subjects research that represent unanticipated problems but are not considered adverse events. For example, some unanticipated problems involve social or economic harm instead of the physical or psychological harm associated with adverse events. In other cases, unanticipated problems place subjects or others at increased risk of harm, but no harm occurs. Examples of unanticipated problems provided by OHRP:

- Clinical trial data are stored on a laptop computer without encryption, and the laptop computer is stolen from the investigator's car on the way home from work.
- As a result of a processing error by a pharmacy technician, a subject enrolled in a multicenter clinical trial receives a dose of an experimental agent that is 10-times higher than the dose dictated by the IRB-approved protocol. While the dosing error increased the risk of toxic manifestations of the experimental agent, the subject experienced no detectable harm or adverse effect after an appropriate period of careful observation.
- Subjects with cancer are enrolled in a phase 2 clinical trial evaluating an investigational biologic product derived from human sera. After several subjects are enrolled and receive the investigational product, a study audit reveals that the investigational product administered to subjects was obtained from donors who were not appropriately screened and tested for several potential viral contaminants, including the human immunodeficiency virus and the hepatitis B virus.

8.4.2. UNANTICIPATED PROBLEM REPORTING

Adverse event as an unanticipated problem
An adverse event, or serious adverse event, also meeting the definition of an unanticipated problem will follow the adverse event reporting pathway by both the investigator and sponsor.

Investigator-identified non-adverse event unanticipated problems
Institutional Review Board. The investigator will report unanticipated problems (UPs) to the Institutional Review Board (IRB) of record as per their policy.

Additional requirements. Other reporting (institutional, local, or state) may be required by the investigator.

Sponsor-identified non-adverse event unanticipated problems
The sponsor-investigator will notify an investigator no less than 1 calendar week after being notified of the unanticipated problem. The investigator will then notify the IRB of record as per their policy as well as follow any other reporting (institutional, local, or state) that may be required.

8.4.3. REPORTING UNANTICIPATED PROBLEMS TO PARTICIPANTS

The sponsor-investigator will review all unanticipated problems and determine the appropriateness of changes to the informed consent document. The sponsor will notify other investigators if re-consenting subjects is mandatory for all subjects or only for those currently undergoing investigational procedures. If the sponsor-investigator does not believe re-consenting is mandatory, other physicians/research team members may request re-consent for all subjects or just those subjects undergoing investigational procedures.

The sponsor- investigator will defer to these IRBs if requested to change the risk section of the informed consent document. Similarly, the sponsor-investigator will also defer to the IRBs if it is determined re-consent should be mandatory.

9. STATISTICAL CONSIDERATIONS

9.1. STATISTICAL HYPOTHESES

The hypothesis is that one of the two ferumoxytol dosing frequencies is generally safe for further efficacy testing.

9.2. SAMPLE SIZE DETERMINATION

This clinical trial utilizes a standard dose-limiting toxicity stopping rule strategy, where cohorts of up to 6 participants will be evaluated to determine the maximum dosage frequency. Six participants must be evaluated at the recommended phase two dosage frequency. Each cohort will treat a maximum of 6 participants. If the starting cohort (Cohort 1) is the RP2D, only 6 evaluable participants are required. If Cohort 2 is the RP2D, then 12 evaluable participants are required. With a 25% screen/withdraw rate, the anticipated number of consented participants is 16.

9.3. POPULATIONS FOR ANALYSES

The safety population includes all patients who received at least one dose of combined therapy (ferumoxytol, pharmacologic ascorbate, temozolomide, radiation). The safety population will be the analysis population for the safety analyses.

9.4. STATISTICAL ANALYSES

9.4.1. GENERAL APPROACH

Continuous variables will be summarized using descriptive statistics such as mean, standard deviation, median, minimum, and maximum. Categorical variables will be summarized by count and proportion.

9.4.2. ANALYSIS OF THE PRIMARY EFFICACY ENDPOINT(S)

The primary endpoint of this study is the frequency of ferumoxytol related-DLT in GBM subjects receiving concurrent pharmacological ascorbate with radiation and temozolomide. DLTs will be recorded and summarized by treatment type occurrence (during radiation and temozolomide or during temozolomide).

Two ferumoxytol dosing schedules will be considered:

- **Cohort 1 [starting]**. Ferumoxytol (510 mg/infusion) administered once per treatment period starting on treatment day 1. The second ferumoxytol treatment period occurs 5-weeks after treatment day 1. The final

ferumoxytol treatment period is synchronized with the first adjuvant temozolomide and pharmacological ascorbate cycle. A total of 3 infusions will be administered.

- **Cohort 2 [escalation]**. Ferumoxytol (510 mg/infusion) administered twice per treatment period (3 to 8 days between infusions). The second ferumoxytol treatment period occurs 5-weeks after treatment day 1 with the same scheduling window. The final ferumoxytol treatment period is synchronized with the first adjuvant temozolomide and pharmacological ascorbate cycle with the same scheduling window, beginning cycle day 1. A total of 6 infusions will be administered.

Six participants will be enrolled to Cohort 1 to evaluate that dosing schedule. If >2 DLTs are identified in Cohort 1, then the trial will be terminated and both cohort schedules considered unsafe. Otherwise, six patients will be enrolled to Cohort 2 (i.e., escalate). If >2 DLTs are identified in Cohort 2, then its schedule will be considered unsafe, and the Cohort 1 schedule will be considered the recommended phase 2 dosing (RP2D). Otherwise, the Cohort 2 schedule will be the RP2D.

The probability of selecting the ferumoxytol dosing schedules is given in the table below as a function of true DLT rates. The study is designed to have high probability of selecting treatment schedules when the true SAE rate is 25% or less. At that rate, there is a 14.1% chance of selecting schedule 1, 69.0% of selecting schedule 2, and 16.9% of incorrectly selecting neither. Conversely, if the DLT rate is 50% for both schedules, then the trial is designed to have a 65.6% chance of declaring them unsafe.

True DLT Rates		**Selection (≤ 2 of 6) Probabilities**		
Cohort 1	Cohort 2	Cohort 1	Cohort 2	Neither
25%	25%	14.1%	69.0%	16.9%
25%	50%	54.5%	28.6%	16.9%
50%	50%	22.6%	11.8%	65.6%

9.4.3. ANALYSIS OF THE SECONDARY ENDPOINT(S)

A formal analysis of secondary endpoints is not planned. Descriptive statistics will be used as appropriate.

9.4.4. SAFETY ANALYSES

The incidence of treatment-emergent adverse events will be summarized by system organ class and/or preferred term, type of adverse event, severity (based on NCI CTCAE v5 grades), and relation to study treatment using the safety population. The most severe grade per patient will be reported. Elapsed days of toxicity will be summarized with descriptive statistics. Dose limiting toxicities as well as adverse events leading to premature discontinuation from the study intervention and serious treatment-emergent adverse events will be presented in tabular form.

9.4.5. BASELINE DESCRIPTIVE STATISTICS

Descriptive statistics will be presented to characterize the patient population. No inferential statistics will be conducted in this safety trial.

9.4.6. PLANNED INTERIM ANALYSES

Interim analyses will be performed to determine safety of the assigned cohort by assessing dose limiting toxicity and to confirm escalation rules. In general, an evaluation is performed after 3 evaluable subjects have been assigned to a cohort and have completed the DLT window. A final interim analysis will be performed on dose limiting data to confirm the recommended phase 2 dose.

9.4.7. SUB-GROUP ANALYSES

Not applicable

9.4.8. TABULATION OF INDIVIDUAL PARTICIPANT DATA

This protocol will comply with section 2038 of the 21st Century Cures Act and supply participant level data as required. De-identified individual-level participant data will be supplied for:

- Gender (self-declared by participant)
- Race
- Ethnicity
- Age at enrollment (in years)

These data will be provided as required in the NIH progress reports.

9.4.9. EXPLORATORY ANALYSES

Exploratory analyses include describing changes in patient-reported outcome measures (PROM). Changes will be summarized with descriptive statistics and presented with plots.

10. SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

10.1. REGULATORY, ETHICAL, AND STUDY OVERSIGHT CONSIDERATIONS

10.1.1. INFORMED CONSENT PROCESS

10.1.1.1. CONSENT/ASSENT AND OTHER INFORMATIONAL DOCUMENTS PROVIDED TO PARTICIPANTS

Elements of Consent
All elements as required under Federal law will be included in the informed consent document. No elements will be waived. Consent forms will be IRB approved.

Assent
Assent will not be used.

Legally authorized representative
To comply with ICH Good Clinical Practice (E6 R2), those who cannot provide independent consent will not be included, as the research goals can be met apart from this population.

Translation(s)
Translated documents and interpreters will not be used for this trial.

Consent/assent and Other Informational Documents Provided to participants
Consent forms describing in detail the study intervention, study procedures, and risks are given to the participant and written documentation of informed consent is required prior to starting intervention/administering study intervention.

10.1.1.2. CONSENT PROCEDURES AND DOCUMENTATION

Consent is obtained from those authorized in the research team (as listed in the approved IRB application and/or delegation of authority log). The consent discussion will take place in a private examination or consult room in the clinic or in the patient's room on the inpatient unit. The potential study participant is provided as much time as needed to review the consent document before a response is required and is encouraged to take the document home for review. Additional copies are provided for family, caregivers, and/or friends as appropriate. Contact information for the research team (typically, the principal investigator, the treating physician(s), and the research nurse(s)) is provided for follow-up questions.

The consenting party asks for full understanding by the potential subject throughout the consent process. The consenter asks the potential subject if there are any questions during the oral description of the study and asks for verbal acknowledgement of full understanding of the study procedures and the general consent elements after a thorough oral presentation of the consent document and after the subject has had time to read it fully and discuss with relatives and friends if desired. The consenter will not attempt to obtain consent if there is any inclination of reduced cognitive ability or concerns of a relative or friend regarding the subject's ability to give consent to the study procedures.

Both the participant and the consenting party will sign the informed consent document consistent with local and Federal requirements. A copy of the signed informed consent document will be provided to the participant. The original signed document will be stored as source document in the participant's case history.

10.1.2. STUDY DISCONTINUATION AND CLOSURE

When a study is prematurely terminated, refer to **Section 7, Study Intervention Discontinuation and Participant Discontinuation/Withdrawal**, for handling of enrolled study participants.

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, the investigator, the funding agency, the Center for Drug Evaluation and Research (CDER) of FDA, the IRB(s) of record, and the Data and Safety Monitoring Committee (DSMC) overseeing this study. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and/or Food and Drug Administration (FDA).

For procedures if the study is halted or prematurely terminated, please refer to Protocol 6.7 as a guideline for safety procedures and final data collection.

10.1.3. CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s) and their interventions. This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), regulatory agencies or pharmaceutical company supplying study product may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at each clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the University of Iowa's Holden Comprehensive Cancer Center using the clinical trials management system OnCore™ (Forte Research Systems, Madison WI). This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by clinical sites and research staff will be secured and password protected. At the end of the study, all study databases will be de-identified and archived at the University of Iowa.

10.1.4. FUTURE USE OF STORED SPECIMENS AND DATA

Participants have the option to permit their medical images, blood and tumor tissue to be stored for future use and to be shared with other researchers. A tracking log is utilized to track those participants who opt-in.

Data collected for this study will be analyzed and stored at the University of Iowa (Iowa City, IA). After the study is completed, for those participants who opted-in to archiving their information, data will be de-identified and archived at the University of Iowa. Researchers interested in using these data will be required to sign a data-use agreement, which limits further secondary analysis as per the Revised Common Rule (January, 2018).

With the participant's approval and as approved by local Institutional Review Boards (IRBs), de-identified biological samples will be stored at the University of Iowa with the same goal. These samples could be used to research the causes of cancer, its complications and other conditions for which individuals with genetic mutations are at increased risk, and to improve treatment. The University of Iowa will also be provided with a code-link that will allow linking the biological specimens with the phenotypic data from each participant, maintaining the blinding of the identity of the participant.

The individual participant can choose to withdraw consent to have biological specimens stored for future research at any time. If research with the blood, tissue, or medical imaging has already been completed, the information from that research may still be used.

10.1.5. KEY ROLES AND STUDY GOVERNANCE

Sponsor-Investigator	Co-chair, radiation therapy	Co-chair, medical oncology
Bryan G. Allen, MD, PhD	John M. Buatti, MD	Varun Monga, MD
Department of Radiation Oncology	Department of Radiation Oncology	Department of Internal Medicine
The University of Iowa	The University of Iowa	The University of Iowa
200 Hawkins Drive, Iowa City IA	200 Hawkins Drive, Iowa City IA	200 Hawkins Drive, Iowa City IA
319-356-3693	319-356-2699	
bryan-allen@uiowa.edu	john-buatti@uiowa.edu	varun-monga@uiowa.edu

Biostatistician	Regulatory Manager	DSMC chair
Brian Smith, Ph.D.	Kellie Bodeker, Ph.D.	Douglas Laux, M.D.
Department of Biostatistics	Department of Radiation Oncology	Holden Comprehensive Cancer Ctr.
The University of Iowa	The University of Iowa	University of Iowa Hospitals & Clinics
145 N. Riverside Dr., Iowa City IA	200 Hawkins Drive, Iowa City IA	200 Hawkins Drive, Iowa City IA
(319) 384-9425	(319) 384-9425	
brian-j-smith@uiowa.edu	kellie-bodeker@uiowa.edu	douglas-laux@uiowa.edu

10.1.6. SAFETY OVERSIGHT

Safety oversight will be under the direction of a Data and Safety Monitoring Committee (DSMC) composed of individuals with the appropriate expertise. A current roster is available on-line at **https://uihc.org/data-and-safety-monitoring-committee**. This committee's membership and policies have been reviewed and approved by the National Cancer Institute under the Holden Comprehensive Cancer Center's Cancer Center Support Grant and Comprehensive Cancer Center designation. Members of the DSMC should be independent from the study conduct and free of conflict of interest, or measures should be in place to minimize perceived conflict of interest. For this reason, an independent reviewer has been designated for this study (William McGinnis, MD). The DSMC will meet at least semiannually to assess safety and efficacy data on each arm of the study. The DMSC operate under the rules of the NCI-approved charter. Key & critical data elements the DSMC have been clearly defined and are provided (10.1.8). The DSMC will provide its input to the IND Sponsor, the IND medical monitor and – as appropriate – the IRB of record, the FDA, the NCI, and/or the responsible funding agency.

10.1.7. CLINICAL MONITORING

Clinical site monitoring is conducted to ensure that the rights and well-being of trial participants are protected, that the reported trial data are accurate, complete, and verifiable, and that the conduct of the trial is in compliance with the currently approved protocol/amendment(s), with International Conference on Harmonisation Good Clinical Practice (ICH GCP), and with applicable regulatory requirement(s).

- Monitoring for this study will be performed by the Holden Comprehensive Cancer Center's Data and Safety Monitoring Committee per the approved, filed plan with the National Cancer Institute.
- The trial's research records will be monitored at minimum twice per year with a minimum of 25% of subjects monitored for the entire study participation (from enrollment to completion of study intervention).
- The investigator will be provided copies of monitoring reports within 14 days of visit / review.
- Details of clinical site monitoring are documented in a Clinical Monitoring Plan (CMP) on file with the Holden Comprehensive Cancer Center. The CMP describes in detail who will conduct the monitoring, at what frequency monitoring will be done, at what level of detail monitoring will be performed, and the distribution of monitoring reports.

- Independent audits will be conducted by the DSMC's designee or the sponsor's designee to ensure monitoring practices are performed consistently across all participating sites and that monitors are following the CMP.

10.1.8. QUALITY ASSURANCE AND QUALITY CONTROL

> The purpose of a sponsor's audit, which is independent of and separate from routine monitoring or quality control functions, should be to evaluate trial conduct and compliance with the protocol, SOPs, GCP, and the applicable regulatory requirements.
>
> To preserve the independence and value of the audit function, the regulatory authority(ies) should not routinely request the audit reports. Regulatory authority(ies) may seek access to an audit report on a case by case basis when evidence of serious GCP non-compliance exists, or in the course of legal proceedings.
>
> ~ International Conference on Harmonisation, *E6*

The sponsor intends to comply with auditing procedures to evaluate conduct and compliance with protocol, SOPs, GCP, and the DSMP filed with this protocol. Audit findings will be disseminated and discussed at quality meetings by the sponsor.

10.1.9. DATA HANDLING AND RECORD KEEPING

10.1.9.1. DATA COLLECTION AND MANAGEMENT RESPONSIBILITIES

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the site investigator. The investigator is responsible for ensuring the accuracy, completeness, legibility, and timeliness of the data reported.

All source documents should be completed in a neat, legible manner to ensure accurate interpretation of data. All source documents should meet the FDA standard of ALCOA (attributable, legible, contemporaneous, original, and accurate).

Source document worksheets will be used for recording data for each participant enrolled in the study. Data recorded in the electronic case report form (eCRF) derived from source documents should be consistent with the data recorded on the source documents. The study site's electronic medical records, including radiation verify and record systems, will mined for data. The EMR will be considered prime source.

Clinical data (including adverse events (AEs), concomitant medications, and expected adverse reactions data) and clinical laboratory data will be entered into OnCore™ (Forte Research Systems, Madison WI), a 21 CFR Part 11-compliant data capture system provided by The University of Iowa Holden Comprehensive Cancer Center. The data system includes password protection and internal quality checks, such as automatic range checks, to identify data that appear inconsistent, incomplete, or inaccurate. Clinical data will be entered directly from the source documents and monitored to verify against transcription errors.

10.1.9.2. STUDY RECORDS RETENTION

As a study conducted under an investigational new drug application (IND 131022) study records must be maintained until the IND sponsor notifies the investigator in writing that they may be destroyed.

The study site must verify with the IRB of record, and any other Federal and local policies, prior to destroying the study records.

10.1.10. PROTOCOL DEVIATIONS

A protocol deviation is any noncompliance with the clinical trial protocol, International Council on Harmonisation Good Clinical Practice (ICH GCP), or Manual of Procedures (MOP) requirements. The noncompliance may be either on the part of the participant, the investigator, or the study site staff. As a result of deviations, corrective actions are to be developed by the site and implemented promptly.

These practices are consistent with ICH GCP:

- 4.5 Compliance with Protocol, sections 4.5.1, 4.5.2, and 4.5.3
- 5.1 Quality Assurance and Quality Control, section 5.1.1
- 5.20 Noncompliance, sections 5.20.1, and 5.20.2.

It is the responsibility of the site investigator to use continuous vigilance to identify and record deviations within 2 working days of identification of the protocol deviation. A protocol violation – a deviation resulting in harm or increased risk to the subject (or decreased acceptability of risk) must be reported to the IND sponsor within 1 working day. All deviations must be addressed in study source documents. Deviation logs must be provided annually to the IND sponsor. Protocol deviations and violations must be sent to the reviewing Institutional Review Board (IRB) per their policies. The site investigator is responsible for knowing and adhering to the reviewing IRB requirements.

10.1.11. PUBLICATION AND DATA SHARING POLICY

This study is registered on clincialtrials.gov Results will be posted as required by FDAAA 801. Other data sharing, such as archived de-identified datasets, will be shared with other researchers after a data-sharing agreement has been signed.

Publications, including peer-reviewed publications, will be submitted in compliance with ICMJE's requirements. Any publications or presentations will provide only de-identified data to safeguard participant privacy.

10.1.12. CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with The University of Iowa has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.]

10.2. ADDITIONAL CONSIDERATIONS

None.

10.3. ABBREVIATIONS

The list below includes abbreviations utilized in this template. However, this list should be customized for each protocol (i.e., abbreviations not used should be removed and new abbreviations used should be added to this list).

AE	Adverse Event
ANCOVA	Analysis of Covariance
CFR	Code of Federal Regulations
CLIA	Clinical Laboratory Improvement Amendments
CMP	Clinical Monitoring Plan
COC	Certificate of Confidentiality
CONSORT	Consolidated Standards of Reporting Trials
CRF	Case Report Form
DCC	Data Coordinating Center
DHHS	Department of Health and Human Services
DSMB	Data Safety Monitoring Board
DRE	Disease-Related Event
EC	Ethics Committee
eCRF	Electronic Case Report Forms
FDA	Food and Drug Administration
FDAAA	Food and Drug Administration Amendments Act of 2007
FFR	Federal Financial Report
GCP	Good Clinical Practice
GLP	Good Laboratory Practices
GMP	Good Manufacturing Practices
GWAS	Genome-Wide Association Studies
HIPAA	Health Insurance Portability and Accountability Act
IB	Investigator's Brochure
ICH	International Conference on Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDE	Investigational Device Exemption
IND	Investigational New Drug Application
IRB	Institutional Review Board
ISM	Independent Safety Monitor
ISO	International Organization for Standardization
ITT	Intention-To-Treat
LSMEANS	Least-squares Means
MedDRA	Medical Dictionary for Regulatory Activities
MOP	Manual of Procedures
MSDS	Material Safety Data Sheet
NCT	National Clinical Trial
NIH	National Institutes of Health
NIH IC	NIH Institute or Center
OHRP	Office for Human Research Protections
PI	Principal Investigator
QA	Quality Assurance
QC	Quality Control
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SMC	Safety Monitoring Committee

SOA	Schedule of Activities
SOC	System Organ Class
SOP	Standard Operating Procedure
UP	Unanticipated Problem
US	United States

10.4. PROTOCOL AMENDMENT HISTORY

The table below is intended to capture changes of IRB-approved versions of the protocol, including a description of the change and rationale. A Summary of Changes table for the current amendment is located in the Protocol Title Page.

Affected Section(s)	Summary of Revisions Made	Rationale
Amendment 2 to Amendment 3		
Cover	Updated Amendment and date	Housekeeping
Header	Updated Amendment and date	Housekeeping
1.4	Updated post-ASC level	Provided window consistent with clinical visits
1.4	Increased window for diagnostic MRI	Consistent with clinical practice for mail-order temozolomide
1.4	Corrected footnote callout for ascorbate infusions to '*p*'	Housekeeping
1.4	Corrected footnote '*d*' to refer to section 8.1.3.1	Housekeeping
6.1.2.4	Corrected delay in radiation therapy to radiation oncologist discretion to align with clinical practice	Risk to deviating from standard practice without justification; justification not realized under study.
6.1.2.5	Inserted, "If held for ≥ 30 days, subject is removed from study," under **Delay**	Clarification as per the principal investigators
6.1.2.5	Inserted "When ANC is above 1.5x 10^9/L, resume TEMODAR at reduced dose for next cycle."	Housekeeping / clarification
6.1.2.5	Updated USPI to match current version	Housekeeping / GCP
8.1.1.2	Removed 2nd "approximately 4 mL" from baseline ascorbate levels.	Housekeeping
8.1.1.2	Removed blood samples for ROS and exchanged for immunology sample	Shift in exploratory objectives
8.1.3	Removed ASC levels drawn during ADJ and follow-up	Per principal investigators; timepoints not unique to current trial intervention.
8.1.3	Removed ROS sampling	Shift in exploratory objectives.
8.1.3	Inserted sampling for immunology	Shift in exploratory objectives
8.1.3	Research iron levels: removed sample at last ascorbate infusion during RT	Per principal investigators
8.2.3	Removed parenthetical text clarifying timepoints	Housekeeping / clarifications
8.2.4.2	Inserted clarification for ferritin and iron panels at follow-up	Clarification
12.2	Clarified RT objectives for CTV and PTV	Should align to standard planning practices.
12.3	Inserted Compliance Criteria	Oversight in initial submission
12.4	Renumbered 12.3 to 12.4	Housekeeping

Amendment 1 to Amendment 2		
Cover page	Amendment version and date	Housekeeping
Header	Amendment version and date	Housekeeping
Throughout	Change from “de-escalation” to “escalation”	Changed design from de-escalation to escalation
Throughout	Changed starting cohort as lowest cohort	
1.4	Corrected CMP to as per MD preference	Align with standard of care
1.4	Inserted liver function tests (AST, ALT, TBIL, ALP)	Monitoring for liver injury
1.4	Updated iron panel & ferritin draw timings; differentiated between clinical and research	Clarification for timepoints, utilization of iron panel, and information gleaned from panel
1.4	Added hold and call for ferumoxytol infusions	Safety review prior to infusion
1.4 “g”	Updated timing of research ASC & Iron draws	Clarification
1.4 “h”	Changed to “at discretion of medical oncologist:	Align with standard of care
1.4 “k”	Updated timing of ROS research draws	Clarification
1.4 “m”	Deleted “consistent with CMP assessment.”	CMP is only ordered clinically
1.4 “o”	Updated clinical iron panel ordering requirements	Safety review prior to, and post, FMX infusions
1.4 “t”	Updated for hold & call prior to infusion	Safety review prior to FMX infusion
4.1	Corrected interim analysis to be after 6 participants or upon DLT threshold being met	Correction per statistician.
4.3.1	Corrected starting dosage and frequency & dosing regimens	Align to dose escalation
4.3.2	Inserted, “inclement weather,” for reason to hold ascorbate infusions.	Clarification
5.3	Updated lifestyle considerations to include alcohol or acetaminophen use during trial	Safety
6.1.2.1	Inserted hold & call text	Safety
6.1.2.1	Corrected monitoring parameters to remove objective thresholds; shift to medical monitoring.	Safety
6.1.2.1	Deleted table 1 per Escalation Rules	Included in error
6.1.2.1	Defined DLT window to begin after one dose of each agent is received (FMX, ASC, RT, TMZ)	Clarification
6.1.2.1	Struck threshold for grade 3 AST / ALT elevation	Covered by serious adverse event / other important medical event if the grade 3 event is considered dose limiting; allows contextual considerations.
6.1.2.1	Clarified Iron Overload definition for purposes of protocol.	Clarification from Hepatology; reviewed by DSMC.
6.1.2.2	Inserted infusion rate	Omitted in error
6.1.2.2	Hold and call for grade 3 AST / ALT inserted.	Safety
6.1.2.3	Inserted “or grade 3” for withholding at physician discretion for non-hematologic reactions.	As per standard of care; physician discretion
6.1.2.3	Struck compliance	Addressed in section 6.4
6.4.1	Inserted requirements for evaluability.	As per peer-reviewed literature; 80% is standard threshold.
8.1.3	Updated timing of Ascorbate Levels, ROS, and research (non-clinical) iron levels.	Clarifications.
8.1.3.1	Updated and clarified T2* imaging per cohort.	Clarification; transition to escalation design
8.2.3	Updated labs to align to schedule of assessments	Clarification; safety
8.2.5	Reduced long-term follow-up to 2 years	Align with grant award
9.4.2	Updated statistical analysis plan	Align with transition to escalation design

Amendment 0 to Amendment 1		
Cover	Updated amendment and date	Housekeeping
Header	Updated amendment and date	Housekeeping
1.2	Clarified order of screening procedures	Housekeeping
1.3	Clarified order of screening procedures	Housekeeping
1.4	Removed "X" from BP for day 0	Error; BP not required at timepoint
1.4	Added "*c*" to BP for BASE timepoint	Error; BP required pre/post 15g test dose
1.4	Edited footnote "g" for iron test collection during course of radiation therapy	Clarification
1.4	Added footnote "t" and assigned to iron panel	Clarification for iron panel draw cycle 1
8.1.3	Synchronized ascorbate levels to iron panel	Clarification
8.1.3.1	Inserted, "sequence details are provided in schema"	Clarification
8.1.3.1	Heavily edited text to clarify order of T2* imaging and associated iron draw procedures.	Clarifications
8.2.2	Corrected "day 1" to "day 0"	Clarification
Version 0.4 to Amendment 0		
Cover	Updated version	Housekeeping
1.2	Added MRI to T2* in Cycle 1 for cohort schemas	IRB requested edit
1.4	Added "MRI" to T2* in footnote a	IRB requested edit
Version 0.3 to Version 0.4		
Cover	Updated version	Housekeeping
Cover	Adding funding instate	New information
1.2	Updated schema to include 15g test dose	Typographical error
1.3	Updated schema to include 15g test dose	Typographical error
SOA	Added footnote 'p' clarifying 15g test dose	Clarification
SOA	Added footnote 'r' clarifying that the initial patient reported outcome measures must be done using a paper form	Clarification
SOA	Added footnote 's' clarifying baseline T2 MR timing	Clarification
6.1.2.1	Added text that radiation must be the next calendar day with a minimum of 2 consecutive fractions in that week.	Clarification.
6.1.2.1	Added text regarding hypersensitivity during ferumoxytol infusion 1	Clarification.
6.1.2.4	Added text that the first week of radiation must have a minimum of 2 consecutive fractions beginning the calendar day after the first ferumoxytol infusion.	Clarification
6.5	Corrected that tumor treating fields may be used after the end of the DLT window (i.e., did state end of cycle 1; should be end of cycle 2).	Error.
8.1.1.2	Deleted ferumoxytol infusion text	Ferumoxytol is not a screening step; error.
8.1.1.2	Added text regarding the order of 15g test dose, eligibility, and the first ferumoxytol infusion.	Clarification.
8.1.1.2	Added text clarifying that T2* MR imaging must be done prior to 15g test dose.	Clarification.

8.1.3.1	Clarified that baseline imaging for T2* does not need to be day 0 but post-consent prior to 15g test dose.	Clarification.
8.2.3	Changed timing for ferritin & iron panel during radiation	Align to Schedule of Assessments and other assessments.
Version 0.2 to Version 0.3 [version 0.2 was submitted to FDA; version 0.3 accepted by FDA]		
Cover	Updated version	Housekeeping
1.4	Added pregnancy test	Housekeeping
1.4	Harmonized concomitant medication review to timing of physical exam	Housekeeping, ease of workflow
1.4	Added timeframe for baseline iron panel	Typographical error / omission
1.4	Moved first PROM from d0 to baseline	Typographical error / omission
1.4	Harmonized PROM and solicited AEs to timing of physical exam	Housekeeping, ease of workflow
1.4	Inserted footnote 'n' and assigned to medical history and solicited AEs	Harmonize to protocol section 8.2.2
1.4	Inserted footnote 'o' and assigned to Iron Panel	Clarify assessments for iron overload evaluation
4.3.1	Corrected first ferumoxytol infusion as defining day 0	Typographical error
5.3	Corrected from "start of study day 1…" to "start of study day 0…" to align with study calendar.	Typographical error
6.1.2.1	Corrected first ferumoxytol infusion as defining day 0	Typographical error
6.1.2.1	Inserted section on monitoring during ferumoxytol treatment, concomitant medication review, procedures to evaluate for suspected iron overload and treatment for iron overload	Clarification requested by FDA / CDER
6.1.2.1	Inserted iron overload as DLT and specified definition for purposes of protocol.	Oversight / inadvertently omitted
6.1.2.2.	Inserted text regarding 15g screening dose	Oversight / inadvertently omitted
6.1.2.2	Clarified 87.5g as treatment dose	Clarification
6.1.2.3	Corrected "grams" to "mg"	Typographical error
6.1.2.5	Inserted dose may be rounded to mg at the discretion of treating physician	Housekeeping
8.1.1.1	Clarified first day of treatment as treatment day 0	Typographical error
8.1.1.2	Inserted text regarding assessments required before 15g asc test dose	Oversight / inadvertently omitted
8.2.3	Clarified physical exam timing (radiation vs. adjuvant)	Clarification and workflow
Header	Updated protocol version and date	Housekeeping

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12. APPENDIX 1: RADIATION THERAPY GUIDELINES

12.1. IMAGING FOR RADIATION THERAPY (I.E., INITIAL SIM AND BOOST)

Imaging per standard practice. Boost field imaging should be completed at approximately 4 weeks (~ 36 Gy) into initial field treatment. If a participant is unable / unwilling to have MRI, CT may be substituted.

- **Steroid**. Record the current steroid dose at each scan.
- **MRI**: Axial T1 and T2 volumetric images with gadolinium contrast will be ordered for the subject for radiation therapy planning purposes.
- **CT**-simulation should be ordered consistent with clinical practice.
- **Scheduling**: Imaging for boost planning will be obtained between radiation fractions 18 and 25.
- **Spectroscopy** (MRS). MRS should be ordered with sim and boost MRI.
- **T2*/QSM**. MR T2*/QSM imaging sequences should be added to radiation therapy planning MRI fields

12.2. RADIATION THERAPY PLANNING

Radiation must begin within 6-weeks post-surgery. Radiation should be prescribed to a total dose of 60 or 61.2 Gy in 30 or 34 fractions (1.8 or 2 Gy/fraction/day). Radiation will be delivered 5 days per week, per normal clinical schedule, and allow holiday & weekend exceptions. The radiation dose will be prescribed to target volume as follows:

- **CTV1**. Clinical Target Volume 1 is defined as the T2 weighted morphologic tumor volume. This volume will receive a radiation dose of approximately 46 Gy in 23 fractions (2 Gy/fx) or 45 Gy in 25 fractions (1.8 Gy/fx).
- **CTV2**. Clinical Target Volume 2 is defined as the T1 enhancing morphologic tumor volume defined in the boost field imaging. This volume will receive approximately 16 or 16.2 Gy in 8 or 9 fractions. The total radiation dose will be 60 Gy/30 fx or 61.2 Gy/34 fx.
- **PTV**. Minimum margin from the edge of GTV for PTV expansion is 8 mm. Larger margins may be necessary and will be at the discretion of the treating radiation oncologist depending on the quality of the planning MR and CT scan(s) and external and internal immobilization.

12.3. COMPLIANCE CRITERIA

Compliance criteria are based upon NRG-BN007.

12.3.1. TARGET VOLUME CONSTRAINTS AND COMPLIANCE CRITERIA

Name of Structure	Dosimetric parameter	Per protocol	Variation Acceptable
PTV (initial field)	D95%	≥ 45	≥ 43.7
PTV (composite)	D95%	59.25-60.75	59.25-60.75
	D10%	≤ 63 Gy	≤ 65.12 Gy
	D0.03cc (Gy)	≤ 64 Gy	≤ 66 Gy

12.3.2. NORMAL STRUCTURE CONSTRAINTS AND COMPLIANCE CRITERIA

Name of Structure	Dosimetric parameter	Per protocol	Variation Acceptable
BrainStem	D0.03cc (Gy)	≤ 55 Gy	≤ 60
Optic Nrv L, or, OpticNrv R	D0.03cc (Gy)	≤ 55 Gy	≤ 60
OpticChiasm_PRV	D0.03cc (Gy)	≤ 55 Gy	≤ 60
Brain		≤ 65	≤ 67
Retina_L or Retina_R	D0.03cc (Gy)	≤ 45	≤ 50
Lens_L or Lens_R	D0.03cc (Gy)	≤ 7	≤ 10
Glnd_Lacrimal_L or Glnd_Lacrimal R	D0.03cc (Gy)	≤ 40	≤45
Cochlea_L or Cochlea_R	Mean dose	≤45	≤50
SpinalCord	D0.03cc (Gy)	≤50	N/A

12.3.3. DELIVERY COMPLIANCE CRITERIA

	Per Protocol	Variation Acceptable
Start date	≤ 6 weeks after surgery	≤ 6 weeks after surgery
Overall treatment time	≤ 46 days	47 – 49 days
Interruptions	≤ 4 days	5 – 7 days

12.4. RADIATION THERAPY

- Radiation will be delivered 5 days per week, per normal clinical schedule, and allow holiday & weekend exceptions.
- **Timing**. Radiation must begin within 6 calendar weeks post-surgery (i.e. 42 days).
- **Schedule**. 1.8 Gy to 2 Gy / fraction /day.
- **Initiation**. The first week should have at least 2 fractions of radiation and 2 infusions of ascorbate.
- **Completion**. The last fraction of radiation (i.e. fini) may be on a Monday. If a lone fraction is delivered in one calendar week for RT fini, a minimum of 2 ascorbate infusions should be delivered that calendar week. This is still considered radiation phase of treatment delivery.