PHASE I/II STUDY OF THE TOLERABILITY, SAFETY AND EFFICACY OF LIPOSOMAL CURCUMIN IN COMBINATION WITH RADIATION AND TEMOZOLOMIDE IN PATIENTS WITH NEWLY DIAGNOSED HIGH-GRADE GLIOMAS

Protocol Number: 1004

Compound: Liposomal Curcumin (LipoCurc)

Study Phase: 1/2

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SIGNATURE PAGE

The signature below provides the necessary assurance that this trial will be conducted according to all stipulations of the protocol, including all statements regarding confidentiality, and according to local legal and regulatory requirements and applicable US federal regulations and ICH E6 Good Clinical Practice (GCP) guidelines.

I agree to conduct the study in compliance with GCP and applicable regulatory requirements.

I agree to conduct the study in accordance with the current protocol and will not make changes to the protocol without obtaining the sponsor's approval and IRB/IEC approval, except when necessary to protect the safety, rights, or welfare of subjects.

Site Investigator Signature:

Signed: Date:

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1 PROTOCOL SUMMARY

1.1 Synopsis

High-grade gliomas (HGG) are the most common adult primary brain cancers and they account for the majority of deaths in patients with gliomas. Glioblastoma, the most common form of primary brain tumor, has the most dismal prognosis.

Current treatment, which includes surgical resection followed by radiation therapy (RT) and temozolomide (TMZ), still results in a median survival of less than 15 months (Stupp et al, 2009).

Temozolomide was approved for use with RT based on a randomized Phase 3 trial comparing TMZ plus RT to RT alone, which led to an improvement in median survival from 12.1 to 14.6 months (Stupp et al, 2005). Since approval of TMZ in 2005, no new drugs have been approved for up-front treatment of HGG. For progressive disease, bevacizumab was approved in 2009, but median survival in this population is still only 9 months (Cohen et al, 2009). Two Phase 3 trials of bevacizumab added to up-front therapy detected an improvement in progression-free survival (PFS) but did not show significant improvement in overall survival (OS) (Chinot et al, 2014; Gilbert et al, 2014). Similarly, other additions to the up-front concurrent chemoradiation (CRT) and adjuvant phase of TMZ treatment have shown an increased likelihood of toxicity without significant prolongation of survival (Stupp et al, 2014; Reardon et al, 2017).

Liposomal curcumin (LC) is a combination of two important treatment modalities. Turmeric, or the plant *Curcuma* spp., has been used orally as a condiment and topically as a medicinal herb for several thousand years in India and Asia. Its active principle, curcumin, was identified in 1820 and its chemical structure, diferuloylmethane, was determined in 1910. In the last 25 years, the development of standardized in vitro and in vivo models of cancers and neuropathic disorders, molecular biology, and genomic tools has elucidated some of the many mechanisms of action of curcumin. Curcumin interacts with multiple cellular constituents resulting in antioxidant and anti-inflammatory activity, enzymatic modulation of cellular signaling systems, and changes in genomic expression patterns. These activities have expanded its potential applications to a variety of systemic diseases and disorders associated with 1 or more components of inflammatory, survival, and replication pathways.

The mechanisms of action of curcumin include antioxidant activity, modulation of disordered proteins, and enzymatic modulation of signaling pathways controlling inflammation, growth, and replication.

Curcumin affects kinases that are products of nononcogenes, but which are essential in tumor cell survival and proliferation. Curcumin's mechanism of action is pleiotropic and in addition to its antioxidant effects; it also modulates multiple signaling pathways commanding survival, repair, and programmed cell death. Curcumin affects intracellular targets that are critical for cell maintenance and growth. These include the proteins of the signal transducer and activator of transcription 3 (STAT-3), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and protein kinase B (AKT) pathways,

which are integrated with additional signaling pathways controlling cancer development. These include anti-apoptosis (B-cell lymphoma-extra large [BCL-xL], survivin), proliferation (tumor necrosis factor [TNF], interleukins, cyclin-D1) tumor promotion (cyclo-oxygenase-2 [COX-2], inducible nitric oxide synthase [INOS], matrix metalloproteinase-9 [MMP-9]), angiogenesis (vascular endothelial growth factor [VEGF]), inflammation (chemokines) and immortality (telomerase). Curcumin acts as an anticancer therapeutic by promoting death pathways and limiting survival pathways in tumor cells (Khor et al, 2006). The most important mechanism of curcumin's activity is suppression of excess tryptophan metabolism through the kynurenine pathway. After stimulation by pro-inflammatory cytokines, indoleamine 2,3 -dioxygenase (IDO) is formed and toxic tryptophan metabolites that are toxic to the brain, heart, and T cells are increased, such as 3-hydroxtkynurenine, 3-hydroxyanthranilic acid, and quinolinic acid. The immunosuppression caused by these toxic kynurenines is especially important in patients with glioblastoma (Sordillo et al, 2017; Sordillo and Sordillo, 2021a; Sordillo and Sordillo, 2021b).

While in vitro and in vivo animal model studies have suggested use of curcumin as a therapeutic agent for systemic diseases, when given orally, it has negligible systemic bioavailability. For this reason, a parenteral liposomal formulation has been developed.

An equally important treatment modality involves the use of our liposome. LC, which is given intravenously (IV) and allows us to achieve curcumin blood levels 2000 times greater than could be achieved with standard oral curcumin (2000 to 4000 ng/mL compared with 1-2 ng/mL with oral curcumin). It protects against cardiac toxicity, including QT prolongation, which would be fatal if not for the protection of the liposome. It also contributes to efficacy. We have shown that derivatives of the liposome have major therapeutic effects in models of numerous diseases (Sordillo and Helson, 2015; Sordillo and Sordillo, 2021a). We have also shown that LC concentrates in malignant cells three to five times more than it does in normal cells (Bolger et al, 2019; Bolger et al, 2021). Curcumin has shown encouraging activity in preclinical cancer models, including in gliomas, especially in combination with cytotoxic drugs.

A full nonclinical safety assessment has been completed for LC. Liposomal curcumin has been shown to be safe and efficacious in animals. The pharmacokinetics (PK), absorption, distribution, metabolism, and excretion (ADME) and toxicology data have not revealed any issues of concern to keep from starting first-in-human (FIH) studies with normal volunteers, as long as potential hemolysis is carefully monitored.

An important benefit from LC is believed to be in combination with cytotoxic chemotherapy and RT (Dhandapani et al, 2007; Aggarwal 2004; Zoi et al, 2021a). We will pursue a Phase 1/2 dose escalation study to assess the maximum tolerated dose (MTD) of LC, in combination with standard RT and TMZ in patients with newly diagnosed HGG, for whom the standard of care is 6 weeks of RT with concomitant TMZ followed by adjuvant TMZ chemotherapy. The study will also assess the toxicity and feasibility of weekly administration of LC in combination with standard therapy, which will provide important information as a basis for design of a future efficacy trial.

Administration of LC has been tested in a Phase 1a and a Phase 1b study in cancer patients (Greil et al, 2018). A recommended phase 2 dose (RP2D) of LC for weekly administration has been established at 300 mg/m^2 IV.

In an FIH safety and PK study in healthy patients conducted at the Medical University in Vienna, LC was well tolerated. The systemic safety of LC was good over the dose range between 10 and 320 mg/m². In patients dosed at 400 mg/m², an increase in mean corpuscular volume (MCV) and erythrocyte echinocyte formation in peripheral venous blood were observed and led to dose de-escalation and additional safety analyses. Minor decreases of erythrocytes and hemoglobin (Hgb) were observed in the 2 patients, but was judged as not clinically significant by the investigator. Markers of hemolysis (hydroxybutyrate dehydrogenase [HBDH], potassium, haptoglobin, lactate dehydrogenase [LDH], erythrocytes, Hgb) did not change significantly.

Blood smears prepared after dose de-escalation revealed transient shape changes of erythrocytes occurring at all investigated dose levels between 120-400 mg/m² (see Investigator's Brochure) that were not connected to clinical symptoms.

Infusion of LC resulted in rapid and dose-dependent development of plasma levels of curcumin and its metabolite tetrahydrocurcumin (THC) with time to maximum plasma concentration (T_{max}) values ranging from 0.9-1.7 hr for curcumin and 0.5-1.8 hr for THC. The maximum plasma concentration (C_{max}) ranged from 42 ± 22 to 2359 ± 412ng/mL for doses between 10 and 400 mg/m², respectively. The plasma levels of curcumin during infusion were 7- to 16-fold higher than those of THC. The clearance of curcumin was similar across doses and ranged from 1.42 to 5.29 L/kg/hr. Steady-state volumes of distribution were variable with dose and ranged from 0.34 to 19.97 L/kg.

The study results support the conclusion that LC, when administered IV, will achieve relevant plasma levels (ng/mL), indicating good systemic availability. Upon termination of infusion, the plasma levels dropped very rapidly for curcumin and THC, with mean residence times over a dose range of 120-400 mg/m^2 ranging from 0.18-0.62 hr for curcumin and 0.58-1.69 hr for THC. The post-infusion plasma exposure of curcumin was 4% to 10% of exposure during infusion, while the post-infusion plasma exposure of THC was 26% to 37% of the exposure during infusion.

Urinary clearance calculated from urine curcumin levels and urine volumes account for a very small percentage (0.12%) of the total systemic clearance of curcumin in humans, consistent with studies in other species.

Protocol Title: Phase I/II Study of the Tolerability, Safety and Efficacy of Liposomal Curcumin in Combination With Radiation and Temozolomide in Patients With Newly Diagnosed High-Grade Gliomas

Rationale: The objective of this study is to assess the safety, tolerability, and efficacy of LC in combination with radiotherapy (RT) and TMZ in patients with HGG.

Objectives and Endpoints

Table 1. Objectives and Endpoints

Objective	Endpoint
Primary	
• To determine the MTD/RP2D of LC in combination with RT and TMZ and adjuvant TMZ in newly diagnosed HGG • To determine the safety and tolerability of LC infused IV over 3 hr	• Number of observed DLTs (as defined in Section 4.1)
Secondary	
• To estimate safety and tolerability of LC in combination with standard RT/TMZ and adjuvant TMZ • To determine feasibility of weekly LC infusion as defined as patients being able to complete 80% of the planned doses of LC, 80% of RT and 60% of TMZ within the first 10 weeks of treatment • To assess efficacy	• Safety assessments include SAEs, AEs, findings on physical examination, clinical laboratory findings, and ECGs • Proportion of patients at each dose level who receive at least 80% of the planned infusions of LC 80% of RT and 60% of TMZ within the first 10 weeks of treatment during the first 10 weeks of treatment • OS and PFS at each dose level; PFS based on RANO criteria

AE = adverse event; DLT = dose-limiting toxicity; ECG = electrocardiography; HGG = high-grade glioma; IV = intravenously; LC = liposomal curcumin; MTD = maximum tolerated dose; OS = overall survival; PFS = progression-free survival; RP2D = recommended Phase 2 dose; RANO = Response Assessment in Neuro-Oncology; RT = radiation therapy; SAE = serious adverse event; TMZ = temozolomide

Overall Design:

This study is a Phase 1/2, multi-center, open-label, dose-escalation study in patients with high-grade malignant gliomas. Dose finding will be performed using a time-to-event Bayesian optimal interval (TITE-BOIN) rule-based schema.

Disclosure Statement: This study is an unblinded, sequential treatment intervention employing 3 dose levels.

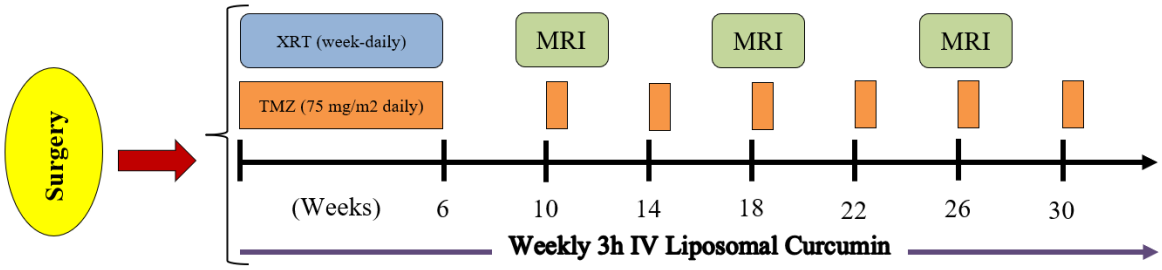
Number of Patients: Approximately 50 patients will be screened to achieve up to 30 patients assigned to study intervention.

Intervention Groups and Duration: The duration of treatment for each patient will be up to 34 weeks. Treatment starts with the beginning of infusion and ends, if tolerated, at the end of Cycle 6 of adjuvant TMZ.

Safety Review Committee: This study will be overseen by a Safety Review Committee (SRC) charged with evaluating dose escalations and monitoring safety data throughout the trial (Section 4.1.6).

1.2 Schema

Figure 1. Study Design



IV = intravenous; MRI = magnetic resonance imaging; TMZ = temozolomide; XRT = radiation therapy

Treatment starts in first week of chemoradiation.

The DLT evaluation period is 10 weeks. Afterward, patients will continue adjuvant TMZ and LC for an additional 24 weeks.

1.3 Schedule of Assessments

Table 2. Schedule of Activities (SoA)

Procedure	Baseline	CRT Period						Post-CRT				Adjuvant Cycles 1 - 6				End Of Treatment[14]	LTFU	End of Study
		Week						Week				Adjuvant Week						
	≤14 Days of D1	1	2	3	4	5	6	7	8	9	10	1	2	3	4			
		D1	D8 ±3	D15 ±3	D22 ±3	D29 ±3	D36 ±3	D43 ±3	D50 ±3	D57 ±3	D64 ±3	D71 ±3	D78 ±3	D85 ±3	D92 ±3		±14 Days	
Informed consent	X																	
Brain MRI[1]	X											X				X		
Inclusion/exclusion criteria	X																	
Demographics and medical history	X																	
Physical and neurological examination	X				X			X				X[5]				X[5]		
Vital signs[2]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
KPS	X			X				X				X				X		
Hematology[3]	X		X	X	X	X	X	X	X	X	X	X				X		
Peripheral smear, haptoglobin[4]	X																	

Procedure	**Baseline**	**CRT Period**						**Post-CRT**				**Adjuvant Cycles 1 - 6**				**End Of Treatment[14]**	**LTFU**	**End of Study**
		Week						**Week**				**Adjuvant Week**						
	≤14 Days of D1	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**1**	**2**	**3**	**4**			
		D1	D8 ±3	D15 ±3	D22 ±3	D29 ±3	D36 ±3	D43 ±3	D50 ±3	D57 ±3	D64 ±3	D71 ±3	D78 ±3	D85 ±3	D92 ±3		±14 Days	
Clinical chemistry[3]	X			X	X			X				X				X		
Complete urinalysis	X																	
Urine pregnancy test (WOCBP only)[6]	X	X						X				X						
12-lead ECG[7]	X				X							X						
Administration of RT[8]		X	X	X	X	X	X											
Administration of TMZ/ondansetron[9]		X	X	X	X	X	X					X						
Administration of LC/premedication[10]		X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Concomitant medications	X[11]	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Adverse events[12]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		X

Procedure	Baseline	CRT Period						Post-CRT				Adjuvant Cycles 1 - 6				End Of Treatment[14]	LTFU	End of Study
		Week						Week				Adjuvant Week						
	≤14 Days of D1	1	2	3	4	5	6	7	8	9	10	1	2	3	4			
		D1	D8 ±3	D15 ±3	D22 ±3	D29 ±3	D36 ±3	D43 ±3	D50 ±3	D57 ±3	D64 ±3	D71 ±3	D78 ±3	D85 ±3	D92 ±3		±14 Days	
Overall survival and progression-free survival[13]																	X	

CRT = chemoradiotherapy; ECG = electrocardiography; Hgb = hemoglobin; IV = intravenous; KPS = Karnofsky Performance Scale; LC = liposomal curcumin; LTFU = long-term follow-up; MRI = magnetic resonance imaging; PO = per os (oral); RT = radiotherapy; TMZ = temozolomide; WOCBP = women of childbearing potential

1. MRI: Performed at baseline (-21 days) prior to first study drug administration, before every odd-numbered adjuvant cycle (1, 3, 5, etc; approximately every 8 weeks), and at the discretion of the Investigator. End of Treatment MRI does not need to be repeated if performed within the 14 days prior to the End of Treatment date.
2. Vital signs: Include systolic and diastolic blood pressure, respiratory rate, heart rate, temperature, weight, and height. Height is required only at the baseline visit. Vital signs must be performed within 120 min of LC administration.
3. Hematology and clinical chemistry performed prior to each adjuvant cycle, and at end of treatment. Can be performed up to 3 days prior to LC administration. End of Treatment assessment does not need to be repeated if performed within 5 days of the last assessment.
4. Assessment of anemia: Peripheral smear and haptoglobin performed at baseline. These tests should be repeated if Hgb decreases 2 g/dL within 1 week or if Hgb < 9 g/dL.
5. Perform assessment prior to every adjuvant cycle. End of Treatment assessment does not need to be repeated if performed within 5 days of the last assessment.
6. Pregnancy test: For WOCBP. Test to be performed within 72 hr prior to first administration of LC, at Week 7, and before every adjuvant cycle.
7. ECG: 12-lead ECG to be performed Week 4 before treatment, then Adjuvant Week 1 before start of each odd-numbered adjuvant cycle (Adjuvant Cycles 1, 3, and 5). Any new clinically relevant finding should be reported as an AE. Males with QTcB interval > 450 msec and females with QTcB

interval > 460 msec should discontinue treatment with LC. Any patient with an increase from baseline in the QTcB interval > 15 msec should discontinue LC.

8. RT: ~2 Gy external beam RT, Monday through Friday.
9. TMZ: Given PO, 30 minutes after administration of antiemetic. No food should be taken for 2 hr before and after dosing. CRT period: 75 mg/m^2 daily during RT; Adjuvant cycles: 150-200 mg/m^2 Days 1 through 5 of each cycle. Patients will be prescribed ondansetron at 8 mg to administer 30 minutes prior to chemotherapy ingestion as well as every 8 hr as needed. Patients can additionally be prescribed prochlorperazine at 5-10 mg as needed every 6 hr per Investigator preference.
10. Infusion of LC: Given as 3-hr IV infusion (± 1 calendar day) by gravity infusion (without infusion pump). Dose as determined by assigned or infusion-adjusted cohort (240 mg/m^2, 300 mg/m^2, 350 mg/m^2, or 400 mg/m^2). Premedications to be given are dexamethasone 4 mg IV and diphenhydramine 25 mg IV.
11. Concomitant medications: Record all medications taken within 14 days prior to baseline visit.
12. Adverse events will be collected from the start of treatment and followed for 30 days after the last dose of LC. Patients removed from the study for unacceptable AEs will be followed until resolution or stabilization of the AE.
13. For the first 2 years after the last dose of LC, patients will be followed for overall survival and progression-free survival every 2 months by telephone call, clinic visit, or medical records. After 2 years have elapsed, patients will be followed every 6 months for overall survival and progression-free survival by telephone call, clinic visit, or medical records.
14. End of Treatment: Evaluations are to be performed within 7 days following the End of Treatment date, unless otherwise indicated. Evaluations that do not need to be repeated include KPS and clinical laboratory parameters (if performed within the 5 days prior to the end of treatment date).

1.4 List of Abbreviations

Abbreviation	Definition
ADME	absorption, distribution, metabolism, excretion
AKT	protein kinase B
AE	adverse event
BBB	blood-brain barrier
BCL-xL	B-cell lymphoma-extra large
CBC	complete blood count
CFR	Code of Federal Regulations
CIOMS	Council for International Organizations of Medical Sciences
C_{max}	maximum plasma concentration
CONSORT	Consolidated Standards of Reporting Trials
COX-2	cyclo-oxygenase-2
CRF	case report form
CRT	chemoradiotherapy
CT	computed tomography
CTCAE	Common Terminology Criteria for Adverse Events
CTV	clinical target volume
CYP450	cytochrome P450
DLT	dose-limiting toxicity
DMSO	dimethyl sulfoxide
DRR	digitally reconstructed radiograph
DSMB	Data and Safety Monitoring Board
ECG	electrocardiogram, electrocardiography
eCRF	electronic Case Report Form
EDTA	ethylenediaminetetraacetic acid
eGFR	estimated glomerular filtration rate
FIH	first in human
FLAIR	fluid-attenuated inversion recovery
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GTV	gross target volume
HBDH	hydroxybutyrate dehydrogenase

hERG	human ether-à-go-go
Hgb	hemoglobin
HGG	high-grade glioma
HIPAA	Health Insurance Portability and Accountability Act
HIV	human immunodeficiency virus
HRT	hormonal replacement therapy
IC_{50}	half-maximal inhibitory concentration
ICH	International Council for Harmonisation
ICMJE	International Committee of Medical Journal Editors
IDO	indoleamine 2,3-dioxygenase
IEC	Independent Ethics Committee
IMRT	intensity-modulated radiotherapy
INOS	inducible nitric oxide synthase
IRB	Institutional Review Board
IUPAC	International Union of Pure and Applied Chemistry
IV	intravenous
JAK	Janus kinase
LC	liposomal curcumin
LDH	lactate dehydrogenase
MCV	mean corpuscular volume
MGMT	O(6)-methylguanine-DNA methyltransferase
MMP-9	matrix metalloproteinase-9
MRI	magnetic resonance imaging
MRSD	maximum recommended safe dose
MTD	maximum tolerated dose
MTIC	5-(3-methyltriazen-1-yl)- imidazole-4-carboxamide
mTOR	mammalian target of rapamycin
NF-κB	nuclear factor kappa-light-chain-enhancer of activated B cells
NOAEL	no observed adverse effect level
OS	overall survival
PD	progressive disease
PFS	progression-free survival
PK	pharmacokinetic

PO	per os (oral)
PRN	pro re nata (as needed)
PTV	planning target volume
RP2D	recommended phase 2 dose
RANO	Response Assessment in Neuro-Oncology
RBC	red blood cell
ROS	reactive oxygen species
RT	radiation therapy
S1P	sphingosine-1-phosphate
SAE	serious adverse event
SK	sphingosine kinase
SoA	Schedule of Assessments
SRC	Safety Review Committee
STAT-3	signal transducer and activator of transcription 3
SUSAR	suspected unexpected serious adverse reaction
TITE-BOIN	time-to-event Bayesian optimal interval
THC	tetrahydrocurcumin
TIW	ter in week (three times weekly)
T_{max}	time to maximum plasma concentration
TMZ	temozolomide
TNF	tumor necrosis factor
TTF	tumor=treating field
ULN	upper limit of normal
VEGF	vascular endothelial growth factor
WHODrug	World Health Organization Drug Dictionary
WNT	wingless and INT-1
WOCBP	women of childbearing potential
WSTFT	weighted standardized follow-up time

2 INTRODUCTION

High-grade gliomas (HGG), which include glioblastoma World Health Organization (WHO) grade IV, and grade III anaplastic astrocytomas WHO grade III, as well as diffuse midline gliomas, are the most common adult primary brain cancers and they account for the majority of deaths in patients with gliomas. Glioblastoma, the most common form of primary brain tumor, has the most dismal prognosis. Even after gross total resection, these high-grade, invasive tumors almost universally recur due to microscopic foci of tumor outside the main mass.

Current treatment, which includes surgical resection followed by radiation therapy (RT) and temozolomide (TMZ), still results in a median survival of less than 15 months (Stupp et al, 2009). More aggressive surgery is associated with increased survival, although, due to the infiltrative nature and the natural limitation of resection of brain tissue, progression still occurs in most patients (Marko et al, 2014).

Temozolomide was approved for use with RT based on a randomized Phase 3 trial comparing TMZ plus RT to RT alone, which led to an improvement in median survival from 12.1 to 14.6 months. Since approval of TMZ in 2005, no new drugs have been approved for up-front treatment of HGG. For progressive disease (PD), bevacizumab was approved in 2009, but median survival in this population is still only 9 months (Cohen et al, 2009). Two Phase 3 trials of bevacizumab added to up-front therapy detected an improvement in progression-free survival (PFS) but did not show significant improvement in overall survival (OS) (Chinot et al, 2014; Gilbert et al, 2014). Similarly, other additions to the upfront concurrent chemoradiation (CRT) and adjuvant phase of TMZ treatment have increased the likelihood toxicity without significant prolongation of survival (Stupp et al, 2014; Reardon et al, 2017).

Liposomal curcumin (LC) is a combination of two highly effective treatments. Turmeric, or the plant *Curcuma* spp., has been used orally as a condiment and topically as a medicinal herb for several thousand years in India and Asia. Its active principle, curcumin, was identified in 1820 and its chemical structure, diferuloylmethane, was determined in 1910. In the last 25 years, the development of standardized in vitro and in vivo models of cancers and neuropathic disorders, molecular biology, and genomic tools has elucidated some of the many mechanisms of action of curcumin. Curcumin interacts with multiple cellular constituents resulting in antioxidant and anti-inflammatory activity, enzymatic modulation of cellular signaling systems, and changes in genomic expression patterns. These activities have expanded its potential applications to a variety of systemic diseases and disorders associated with 1 or more components of inflammatory, survival and replication pathways.

The mechanisms of action of curcumin against GBM include antioxidant activity, cytotoxic effects, modulation of disordered proteins, chemo- and radiosensitization, alterations in metabolism of tryptophan, and enzymatic modulation of signaling pathways controlling inflammation, growth, and replication.

Curcumin affects kinases that are products of nononcogenes, but that are essential in tumor cell survival and proliferation. Curcumin's mechanism of action is pleiotropic. In

addition to its antioxidant effects, it modulates multiple signaling pathways commanding survival, repair, and programmed cell death. Curcumin affects intracellular targets that are critical for cell maintenance and growth. These include the proteins of the signal transducer and activator of transcription 3 (STAT-3), nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB), and protein kinase B (AKT) pathways, which are integrated with additional signaling pathways controlling cancer development. These include anti-apoptosis (B-cell lymphoma-extra large [BCL-xL], survivin), proliferation (tumor necrosis factor [TNF], interleukins, cyclin-D1) tumor promotion (cyclo-oxygenase-2 [COX-2], inducible nitric oxide synthase [INOS], matrix metalloproteinase-9 [MMP-9]), angiogenesis (vascular endothelial growth factor [VEGF]), inflammation (chemokines) and immortality (telomerase). Curcumin acts as an anticancer therapeutic by promoting death pathways and limiting survival pathways in tumor cells (Khor et al, 2006).

Cytotoxic effects attributed to curcumin include cell cycle arrest, apoptosis, autophagy, changes in gene expression, and disruption of molecular signaling. Curcumin inhibits growth of gliomas (Zhuang et al 2012; Gersey et al 2017), promotes of cell death in glioma (Dhandapani et al 2007; Zoi et al, 2021a) and may target cancer stem cells in glioma via selective apoptosis or differentiation (Fong et al, 2010; Lim et al, 2011; Sordillo and Helson, 2015; Zoi et al, 2021b; Zoi et al, 2021a; Abdullah Thani et al, 2012; Huang et al, 2019). Disruption of signaling pathways affects the wingless and INT-1 (WNT) pathway (Leow et al, 2010; Zuccarini et al, 2018), as well as NOTCH (Subramaniam et al, 2012), MAPK (Sordillo and Helson, 2015), hedgehog (Elamin et al, 2010; Zuccarini et al, 2018), and Janus kinase (JAK)/STAT (Alexandrow et al, 2012; Gersey et al, 2017).

Curcumin has been shown to increase radiosensitivity (Dhandapani et al, 2007; Aggarwal 2004) and to exhibit synergy with RT in glioma cell lines (Zoi et al, 2021a). As curcumin decreases oxidative stress, it potentiates TMZ by increasing the cytotoxicity of TMZ (Wu et al, 2015) and by generating ROS and suppressing the mammalian target of rapamycin (mTOR) pathway (Yin et al, 2014; Gersey et al 2017; Jagetia 2007). Curcumin increases ceramide production, which may inhibit resistance to TMZ (Moussavi et al, 2006; Grammatikos et al, 2007; Hara et al, 2004).

Decreases in proinflammatory cytokines are observed with administration of curcumin. Pro-inflammatory cytokines such as STAT3 upregulate release of cytokines by gliomas (Nduom et al, 2015). Suppression of STAT3 by curcumin reduces the tumor promoter TGF beta, (Roy et al, 2018; Seystahl et al, 2017; Saidi et al, 2019), as well as TNF-α (Chan 1995; Okunieff et al, 2006; Ryan et al, 2013), IL-1, IL-6 (associated with higher grades and poor survival), IL-8, and indoleamine 2,3-dioxygenase (IDO) (which stimulates excess tryptophan metabolites through the kynurenine pathway, resulting in immunosuppression (Sordillo et al, 2017; Sordillo and Sordillo, 2021a; Sordillo and Sordillo, 2021b). Inhibition of the JAK/STAT3 in glioma (Senft et al, 2010; Piperi et al, 2019). Interruption of the NF-κβ signaling pathway mediates the anti-inflammatory effect of curcumin (Dhandapani et al, 2007), and effects on WNT pathway suppress proliferation of gliomas (He et al, 2014).

Glioblastoma multiforme alters tryptophan metabolism and ceramides. Ceramides promote apoptosis, opposing sphingosine-1-phosphate (S1P) (Obeid et al, 1993; Hannun and Obeid, 1997; Kawamura et al, 2009; Venable et al, 1995; Radeff-Huang et al, 2007; Olivera et al, 1999). The balance ceramides and S1P, mediated by sphingosine kinase (SK), is termed the sphingolipid rheostat (Cuvillier et al, 1996; Paugh et al, 2009; Sordillo et al, 2016) and may be disordered in GBM (Sordillo et al, 2016). Sphingosine kinase levels are increased in GBM (Li et al, 2008; Estrada-Bernal et al, 2011; Anelli et al, 2008), and overexpression of S1P is associated with higher tumor grades and poor survival (Abuhusain et al, 2013; Van Brocklyn et al, 2005; Yoshida et al, 2010; Abuhusain et al, 2013). Tumors respond to treatment by secreting neurotoxins that alter metabolism of tryptophan and inducing resistance to treatment (Yen et al, 2002; Prendergast 2011). Expression of one such molecule, IDO, is associated with higher tumor grades and poor survival (Wainwright et al; 2012; Yu et al, 2018). Shunting of tryptophan metabolism via the kynurenine pathway (Li et al, 2017) leads to formation of products of the kynurenine pathway are toxic to T cells and express mTOR (Badawy 2018; Moon et al, 2015). Curcumin inhibits IDO (Chen et al, 2012; Jeong et al, 2009; Bose et al, 2015).

While in vitro and in vivo animal model studies have suggested use of curcumin as a therapeutic agent for systemic diseases, when given orally, it has negligible systemic bioavailability. For this reason, a parenteral liposomal formulation has been developed.

For clinical evaluation, curcumin is synthesized at Sami Labs Limited (India) under Good Manufacturing Practice (GMP) conditions to 99.2 % purity. To increase bioavailability, a liposomal formulation of curcumin is manufactured at Polymun Scientific GmbH (Vienna, Austria) under GMP.

2.1 Study Rationale

New treatments for patients with high-grade brain cancers are urgently needed as currently available treatment options are scarce and offer only limited benefit to patients. Most anti-cancer drugs are unable to cross the blood-brain barrier (BBB) to reach therapeutic concentrations in the target tissue. The liposomal formulation possesses promising properties that make clinically meaningful delivery across the BBB a real possibility. Curcumin has shown encouraging activity in preclinical cancer models, including in gliomas, especially in combination with cytotoxic drugs.

A full nonclinical safety assessment has been completed for LC. Liposomal curcumin has been shown to be safe and efficacious in animals. The pharmacokinetics (PK), absorption, distribution, metabolism, and excretion (ADME) and toxicology data have not revealed any issues of concern to keep from starting first-in-human (FIH) studies with normal volunteers, as long as potential hemolysis is carefully monitored. A maximum recommended safe starting dose (MRSD) of 10mg/m^2 has been established to ensure a safe and well-controlled Phase 1 clinical trial.

We will pursue a Phase 1 dose-escalation study to assess the maximum tolerated dose (MTD) of LC, in combination with standard RT and TMZ in patients with newly diagnosed HGG, for which the standard of care is 6 weeks of RT with concomitant TMZ,

followed by adjuvant TMZ chemotherapy. The study will also assess the toxicity and feasibility of weekly administration of LC in combination with standard therapy, which will provide important information as a basis for design of a future efficacy trial.

2.2 Background

Administration of LC has been tested in a Phase 1a and a Phase 1b study in cancer patients (Greil et al, 2018). The RP2D of LC for weekly administration has been established at 300 mg/m^2 intravenously (IV).

Initial clinical data show an early drop in plasma levels of LC after discontinuation of IV infusion (Storka et al, 2015); however, drug accumulated at high levels in polymorphonuclear cells and was still detectable several hours after injection. Preclinical data in rat brain show encouraging drug levels in different areas of the normal brain, suggesting that LC can cross the BBB (Chiu et al, 2011).

2.2.1 Efficacy and Safety Pharmacology

Curcumin has been shown to modulate multiple cell signaling pathways and interact with numerous molecular targets, including cell cycle, apoptosis, proliferation, survival, invasion, angiogenesis, metastasis, and inflammation (Goel et al, 2008). Given its ability to affect multiple targets it is believed to have the potential to act against a large number of cancers, inflammatory diseases, parasitic diseases such as malaria, neurodegenerative diseases, arthritic degenerative conditions, cystic fibrosis, and diabetes.

Liposomal curcumin induced a dose-dependent inhibition of in vitro cell growth at concentrations of 2.5 to 100 µM in 6 pancreatic, and 2 colorectal cancer cell lines (Li et al, 2005; Li et al, 2007). Liposomal curcumin inhibited in vivo tumor growth in 2 xenograft mouse models of human pancreatic cancer (Li et al, 2005). It was shown that 20 mg/kg was the minimum effective dose. This is equivalent to 60 mg/m^2. A dosing regimen experiment showed that three times a week (TIW) dosing was the optimal dosing schedule for tumor growth suppression.

During the safety pharmacology evaluation of LC, the curcumin drug substance alone was shown to have inhibitory effects on the human ether-à-go-go (hERG) ion channel with a half-maximal inhibitory concentration (IC_{50}) of 4.9 µM (1800 ng/mL). When the LC drug product was tested, even at the highest concentration tested (11.4 µM; 4200 ng/mL) a 50% inhibition of hERG was not attained. Therefore, for LC, the IC_{50} is above the maximum plasma concentration (C_{max}) thus far observed in the Phase 1 trial. Although there was a signal for inhibition of hERG in vitro at very high concentrations, no effects on electrocardiograms (ECGs) were seen in the dog 4-week toxicity study at doses up to 20 mg/kg (400 mg/m^2). No effects of LC were seen on respiratory or neurological endpoints in the dog 4-week toxicity study.

2.2.2 Pharmacokinetics

The cytochrome P450 (CYP450) studies show no tendency for inhibition or induction of the common CYP450 drug metabolism enzymes, and, therefore, it is not expected that

LC will have major drug-drug interactions. Tetrahydrocurcumin (THC) is a major metabolite that is detected in plasma at high levels. Tissue distribution data obtained in a dog study after 2-hr and 8-hr continuous infusion revealed that 8-hr infusion yields lower concentrations of curcumin and THC in plasma but higher concentrations in target tissues than a 2-hr infusion.

2.2.3 Toxicology

Liposomal curcumin was tested in the in vitro *Salmonella typhimurium* (Ames) assay, and the in vivo mouse micronucleus assay. There were no signals for mutagenic potential from either of these studies.

Rat and a dog 4-week toxicity studies were done. The rats were given IV bolus injections TIW (empty liposomes, and 10, 20 and 40 mg/kg LC). The dogs were given IV infusions at 10 mL/kg/h for 1 h TIW (5% dextrose in water, empty liposomes, and 5 and 20 mg/kg LC).

For the rats, there were no deaths on the study, and no clinical signs. There were no effects on body weight, food consumption, clinical chemistries or organ weights, and no treatment- related effects on gross or microscopic pathology. Red blood cell (RBC) parameters began to decrease for all groups, including the empty liposomes group, with a nadir during Week 2. During Weeks 3 and 4 the RBC parameters began to improve, and 96 hr after the last dose, the parameters were approaching the normal range. Treatment with empty liposomes had the least effect, with a dose-response effect of the LC. Because the effects on the RBCs were minimal with no other adverse effects, the no observed adverse effect level (NOAEL) for the rat was considered to be > 40 mg/kg.

For the dogs treated with 5 mg/kg LC, the analysis of all generated data including clinical observations, ophthalmology, ECGs, clinical pathology, gross necropsy, and histopathology revealed no treatment-related toxicity.

In dogs dosed with empty liposomes during the first infusion, starting within 15-30 minutes of infusion, most animals started showing clinical signs that included lacrimation, labored breathing that progressed into passivity, weakness, and lethargy. During this period, pulse was weak and blood pressure was undetectable (hypotension). These symptoms were transient and lasted for about 20-30 minutes, after which animals started recovering. By the end of the infusion period and/or immediately after the infusion, all dogs recovered. During the subsequent dosing, these symptoms were present in most animals; however, they were of lesser intensity, as if the animals were adapting to the dosing. (Also, in 1 dog dosed with LC at 20 mg/kg on a few occasions during the infusions, there was angioneurotic edema [swollen face, eyelids, lips] and urticaria noted.) This anaphylactoid reaction is common after infusion with liposomes and was not unexpected (Szebeni et al, 2007). It should be noted that this hypersensitivity reaction occurred in all dogs infused with empty liposomes and in only 1 animal dosed with LC at 20 mg/kg. No animals in the group infused with LC at 5 mg/kg were affected. Pretreatment with an antihistamine and a corticosteroid will be performed in the Phase 1 clinical trial, as is usually done with infusion of liposomes. Pretreatment was not included

in this Good Laboratory Practice (GLP) dog toxicity study as to not interfere with the safety assessment of the LC.

For the dogs treated with the high dose of 20 mg/kg group, 2 animals died after the third dose. All animals in this group showed clinical signs of anorexia, significant weight loss, hematuria (3+), pale mucosal membranes, and intravascular hemolysis resulting in severe anemia. This severe hemolysis was seen at 6 hr and 24 hr after end of infusion. This was reflected in decreased RBC counts, hematocrit, hemoglobin (Hgb); and hematuria. Because of the severe hemolysis, the dose was lowered from 20 mg/kg to 10 mg/kg. After the dose was lowered to 10 mg/kg, the decreased RBC parameters in these dogs began to recover even while still being dosed at 10 mg/kg. The RBC parameters continued to improve during the 4-week recovery period. Reticulocytes increased very significantly after the second dose of 20 mg/kg. When the dose was lowered from 20 to 10 mg/kg, reticulocytes recovered significantly, and reached the normal range during the recovery period. Clinical chemistry findings included increases in total bilirubin, phosphorus, lactate dehydrogenase (LDH), and cholesterol.

In vitro hemolytic potential studies were done with both curcumin drug substance and LC. For dogs, hemolysis was seen at the two highest concentrations of LC (27% and 4% hemolysis [3200 and 1600 µg/mL, respectively]). For the human, hemolysis was only observed at the highest concentration (21% hemolysis at 3200 µg/mL). This concentration is 1200-fold the highest C_{max} seen in humans given the highest dose of 400 mg/m^2.

Since (1) the hemolysis began to reverse after changing the high dose from 20 to 10 mg/kg, (2) hemolysis can be easily monitored in the Phase 1 trials, and (3) there were no effects at 5 mg/kg, it was proposed that a 1/10 safety factor be applied for calculation of the MRSD for the Phase 1 trial. A 5 mg/kg dose in the dog is equivalent to 100 mg/m^2. With a safety factor of 1/10, the MRSD for the Phase 1 trial was 10 mg/m^2.

In vitro formation of echinocytes was investigated in ethylenediaminetetraacetic acid (EDTA) blood from two healthy donors incubated with 1 µg/mL, 10 µg/mL, or 100 µg/mL LC at 37°C. At 1 µg/mL, no effect on erythrocytes was observed. At 10 µg/mL and 100 µg/mL, erythrocytes changed from their normal biconcave shape and became spherical and spiked. The peak effect was already detectable at 30 minutes and lasted for the observation period of 4 hr.

Upon incubation with curcumin drug substance dissolved in dimethyl sulfoxide (DMSO) again, at a threshold dose of 10 µg/mL curcumin, erythrocytes changed their shape and became spherical and spiked after an incubation period of 30 minutes. This shape change normalized between 120 and 240 minutes.

When incubated with empty liposomes, the erythrocytes exhibited shape change after 240 minutes at a lipid concentration corresponding to 10 µg/mL LC. Compared to LC, a change in the shape of the RBCs was seen at the same concentration, but the time point of occurrence was delayed. Incubation with sample buffer or 0.99% DMSO did not influence the morphology of the RBC over 4 hr (Storka et al, 2013).

2.2.4 Clinical Studies

2.2.4.1 Phase 1a Study in Healthy Patients (LipoCurc 1001)

An FIH safety and PK study in healthy patients was conducted at the Medical University in Vienna (Principal Investigator Michael Wolzt) between August 2011 and October 2012. Eligible male or female healthy patients were allocated to ascending dose groups starting with the lowest dose and randomized to receive either LC or placebo.

The originally planned dose groups were 10, 20, 40, 80, 120, and 180 mg/m^2 LC suspended in 5% glucose in a total of 500 mL water, and patients received IV premedication with dexamethasone 4 mg and diphenhydramine 30 mg diluted in 100 mL NaCl. Given the excellent tolerability, and following a protocol amendment, another set of patients were recruited to the dose levels 180, 240, 320, and 400 mg/m² with premedication with diphenhydramine alone.

The systemic safety of LC was good over the dose range between 10 and 320 mg/m². In patients dosed at 400 mg/m², an increase in mean corpuscular volume (MCV) and erythrocyte echinocyte formation in peripheral venous blood were observed and led to dose de-escalation and additional safety analyses. Minor decreases of erythrocytes and Hgb were observed in the 2 patients, but were judged as not clinically significant by the investigator. Markers of hemolysis (hydroxybutyrate dehydrogenase [HBDH], potassium, haptoglobin, LDH, erythrocytes, and Hgb) did not change significantly.

Blood smears prepared after dose de-escalation revealed transient shape changes of erythrocytes occurring at all investigated dose levels between 120 and 400 mg/m² (see Investigator's Brochure), which were not connected to clinical symptoms. For patients dosed with 120 mg/m²,the process had a later onset and presumably only less severe changes were induced in the erythrocyte membrane. Eight patients were treated at 120 mg/m². Investigation of MCV, the parameter that triggered the preparation of blood smears, showed no change in the median MCV values (change between baseline and highest value < 1 fL) at 120 mg/m², whereas the median change of MCV for doses 180-400 mg/m² was between 4 and 13 fL.

In light of complete absence of clinical symptoms or clinically relevant changes in laboratory safety parameters, administration of 120 mg/m² LC over 24 hr using a continuous infusion pump seemed to be a safe and well-tolerated starting dose for clinical studies in cancer patients. However, the study was temporarily interrupted after 2 infusions in the first patient due to precipitate formation in the infusion line, most likely caused by shear forces and increased temperature at the pump head. The patient did not experience any adverse events (AEs) related to this incident. Based on the results of subsequent in-use stability tests and a decision by the Data and Safety Monitoring Board (DSMB), the study was continued by administering LC over 8 hr as a gravity infusion (without an infusion pump). As a reduced infusion time increases the concentration of LC over the time, the starting dose was reduced from 120 mg/m² to 100 mg/m². Gravity infusion (without an infusion pump) will be used throughout the clinical trial. The administration system via the syringe pump was tested in compatibility studies and the

stability of LC was demonstrated for 12 hr of perfusion and an extended observation period of 24 hr.

Infusion of LC resulted in rapid and dose-dependent development of plasma levels of curcumin and its metabolite THC with time to maximum plasma concentration (T_{max}) values ranging from 0.9-1.7 hr for curcumin and 0.5-1.8 hr for THC. C_{max} ranged between 42 ± 22 and 2359 ± 412 ng/mL for doses between 10 and 400 mg/m², respectively. The plasma levels of curcumin during infusion were 7- to 16-fold higher than those of THC. The clearance of curcumin was similar across doses and ranged from 1.42-5.29 L/kg/hr. Steady-state volumes of distribution were variable with dose and ranged from 0.34-19.97 L/kg.

The study results support the conclusion that the test medication LC, when administered IV, will achieve relevant plasma levels (ng/mL), indicating good systemic availability. Upon termination of infusion, the plasma levels dropped very rapidly for curcumin and THC, with mean residence times over a dose range of 120–400 mg/m^2 ranging from 0.18-0.62 hr for curcumin and 0.58-1.69 hr for THC. The postinfusion plasma exposure of curcumin was 4% to 10% of exposure during infusion, while the postinfusion plasma exposure of THC was 26% to 37% of the exposure during infusion.

Urinary clearance calculated from urine curcumin levels and urine volumes account for only a very small percentage (0.12%) of the total systemic clearance of curcumin in humans, consistent with studies in other species.

2.3 Benefit/Risk Assessment

High-grade gliomas carry a most dismal prognosis. Even after gross total resection followed by RT and TMZ, the median survival is poor. New therapies are clearly needed for this devastating disease.

Turmeric is well tolerated and is believed to have the potential to act against a large number of cancers by promoting death pathways and limiting survival pathways in tumor cells (Khor et al, 2006).

More detailed information about the known and expected benefits and risks and reasonably expected AEs associated with LC may be found in the Investigator's Brochure.

3 OBJECTIVES AND ENDPOINTS

Table 3. Objectives and Endpoints

Objective	Endpoint
Primary	
• To determine the MTD/RP2D of LC in combination with RT and TMZ and adjuvant TMZ in newly diagnosed HGG • To determine the safety and tolerability of LC infused IV over 3 hr	• Number of observed DLTs (as defined in Section 4.1.1)
Secondary	
• To estimate safety and tolerability of LC in combination with standard RT/TMZ and adjuvant TMZ • To determine feasibility of weekly LC infusion as defined as patients being able to complete 80% of the planned doses of LC, 80% of RT and 60% of TMZ within the first 10 weeks of treatment • To assess efficacy	• Safety assessments include SAEs, AEs, findings on physical examination, clinical laboratory findings, and ECGs • Proportion of patients at each dose level who receive at least 80% of the planned infusions of LC 80% of RT and 60% of TMZ within the first 10 weeks of treatment • OS and PFS at each dose level, PFS based on RANO criteria

AE = adverse event; DLT = dose-limiting toxicity; ECG = electrocardiography; HGG = high-grade glioma; IV = intravenously; LC = liposomal curcumin; MTD = maximum tolerated dose; OS = overall survival; PFS = progression-free survival; RP2D = recommended phase 2 dose; RANO = Response Assessment in Neuro-Oncology; RT = radiation therapy; SAE = serious adverse event; TMZ = temozolomide

4 STUDY DESIGN

4.1 Overall Design

This Phase I/II, dual-center, single-institution, open-label, dose-escalation study will define the MTD/recommended Phase 2 dose (RP2D) of LC, administered IV weekly in combination with standard CRT (60 Gy in 30-33 fractions M-F, and daily oral TMZ 75 mg/m^2), in patients with high grade malignant gliomas that fulfill the eligibility criteria of the study.

This study seeks the MTD/RP2D of LC when added to TMZ during concurrent RT and adjuvant TMZ after RT. The study will evaluate escalating doses of LC delivered by IV infusion weekly as a gravity infusion (without infusion pump). Within each cohort, the dose will remain the same. In the first cohort, dosing will begin at Level 1 (300 mg/m^2). The infusion of LC will begin at the start of CRT. Patients will be evaluable for the cohort if they have completed 80% of the planned doses of LC, 80% of RT and 60% of TMZ within the first 10 weeks of treatment. Patients who experience a dose-limiting toxicity (DLT) will be evaluable for the cohort if they have received at least 1 dose of LC.

4.1.1 Dose-Limiting Toxicities

The DLT assessment period for this study is 10 weeks: 6 weeks of concurrent LC and CRT, and 4 weeks of single-agent LC.

Hematological Toxicities

Hematological toxicities will be considered dose limiting if any of the following occur and complete blood counts (CBCs) and differentials were obtained according to the mandated schedule (CBC, differential, and platelet count drawn twice a week until the ANC ≥ 1500/ μL and platelet count ≥ 100,000/μL):

- ANC < 500/μL
- Platelet count < 25,000/μL
- Grade 4 anemia
- Grade 3 hemolysis
- Febrile neutropenia
- Any hematological toxicity that prevents administration of ≥ 80% of the planned LC dose

Grade 3 or 4 lymphopenia will not be considered a DLT.

Central Nervous System Toxicities

Central nervous system (CNS) toxicities are:

- ≥ Grade 2 CNS ischemia

- ≥ Grade 2 neurological toxicities that interfere with activities of daily living and do not resolve spontaneously or with steroids, anticonvulsants, or electrolyte correction within 2 weeks
- Symptomatic CNS hemorrhage of any grade

Nonhematological, Non-CNS Toxicities

Grade 3 and 4 nonhematological, non-CNS toxicities will be considered DLTs, with the following exceptions:

- Grade 3 nausea, vomiting, or diarrhea despite sufficient prophylaxis with a duration < 3 days
- Alopecia
- Grade 3 fatigue
- Grade 3 hyperglycemia that is reversible and without clinical symptoms
- Grade 3 electrolyte disturbances that are asymptomatic and reversible within 3 days, and do not re-occur upon continuing or re-initiation at the same dose
- Grade 3 or 4 hypophosphatemia, unless considered clinically relevant
- Grade 3 or 4 elevations in alkaline phosphatase
- Grade 3 hypertension that resolved to ≤ Grade 2 hypertension within 1 week either spontaneously or upon implementation of antihypertensive therapy
- A first episode of deep venous thrombosis (DVT) or pulmonary embolism

Hepatic DLTs are:

- ALT or AST > 8*upper limit of normal (ULN), regardless of duration
- ALT or AST >5*ULN and ≤ 8*ULN that fails to return to ≤ Grade 1 within 2 weeks despite medical intervention
- Total bilirubin > 5*ULN
- ALT or AST > 3*ULN and concurrent total bilirubin > 2*ULN

FOR PATIENTS ON CONCURRENT TREATMENT, ANY DLT (AS DEFINED ABOVE) CAUSING DELAY IN TREATMENT OF OVER 7 DAYS WITHOUT RECOVERY TO A ≤ GRADE 1 OR BASELINE STATUS WOULD RESULT IN TAKING THE PATIENT OFF CONCURRENT TREATMENT.

FOR PATIENTS RECEIVING ADJUVANT TREATMENT, ANY AE (AS DEFINED ABOVE FOR DLT) CAUSING DELAY IN TREATMENT OF OVER 14 DAYS (POST CYCLE 1 DAY 1) WITHOUT RECOVERY TO A ≤ GRADE 1 OR BASELINE STATUS WOULD RESULT IN TAKING THE PATIENT OFF TREATMENT.

4.1.2 Dose Escalation

Following tumor resection, eligible patients who have signed the Informed Consent Form (ICF) will be assigned a dose of LC.

There will be a maximum of 4 dose levels assessed in this study. The first cohort of patients will start at Dose Level 1 (Table 4). If indicated based on the number of observed DLTs at Dose Level 1, patients may be enrolled in Dose Level -1.

Table 4. Liposomal Curcumin, Radiotherapy, and Temozolomide Dose Escalation Schedule

Dose Level	Dose	
	LC	TMZ
Level -1	240 mg/m^2	75 mg/m^2 concurrent RT 150-200 mg/m^2 Cycles 1-6 adjuvant
Level 1 (Starting Dose)	300 mg/m^2	
Level 2	350 mg/m^2	
Level 3	400 mg/m^2	

LC = liposomal curcumin; RT = radiotherapy; TMZ = temozolomide

The decision whether a new patient should be assigned to the current dose level or to a higher or lower dose level (escalation or de-escalation) is a function of the number of patients already treated at the current dose level, the number of patients who experienced a DLT at the current dose level, and the number of patients who have neither experienced a DLT nor completed their 10-week DLT evaluation period. The Safety Review Committee (SRC) will oversee dose level decisions throughout the dose escalation phase of the study.

All participating sites are expected to notify the Sponsor when a DLT has occurred.

The study will enroll and treat patients in cohorts of 3 in the dose escalation phase. The first cohort will receive 300 mg/m^2 (Dose Level 1) of LC weekly. The target DLT risk is 30%. During the assessment window, planned doses will be dropped if the data suggest a $\geq$ 95% probability that the dose will produce a DLT risk > 30%. Using the proposed time-to-event Bayesian optimal interval (TITE-BOIN) design, enrollment in the escalation phase will stop when 12 patients are assigned to the same dose level or when a total of 24 patients have been treated, whichever happens first.

Once the MTD is determined, an additional 6 patients will be treated in an expansion cohort at this dose level or at a lower dose level determined to be the RP2D. For the expansion cohort, patients will continue to be monitored for occurrence of DLTs. If 2 of the first 5 patients or if $\geq$ 2 of 6 patients experience a DLT, the Principal Investigator will discuss with all study Investigators and with the Medical Monitor and/or SRC whether further addition of patients is needed to re-assess the MTD. Monitoring of all safety and toxicity data will be done by the Principal Investigator and the SRC on a real-time basis.

In the absence of treatment delays due to AEs, treatment for an individual patient may continue until the completion of standard therapy (until the end of Adjuvant Cycle 6) or until one of the following criteria applies:

- Disease progression

- Intercurrent illness that prevents further administration of treatment
- Unacceptable AEs
- Patient decides to withdraw from the study
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the Investigator
- Clinical progression
- Patient noncompliance
- Pregnancy
- Termination of the study by the Sponsor
- The drug manufacturer can no longer provide the study agent

Toxicities requiring dosing delay or modifications will result in dose adjustments as outlined in Section 5.10 (the minimum dose is 240 mg/m^2).

Additional details of the study design and escalation decisions are in Section 9.

4.1.3 Administration of Study Therapies

- Treatments will be weekly 3-hr infusions of LC via gravity infusion (without infusion pump) at the assigned dose through concurrent CRT and the 4-week post-CRT recovery time, at which point the DLT evaluation period is completed.
- Patients will continue weekly LC infusions through the adjuvant TMZ treatment period (150-200 mg/m^2 on Weeks 1-4 of each 28-day cycle) for a maximum of 24 additional weeks (ie, up to 6 additional 4-week cycles).

4.1.4 Clinical Sites

Treatment will be administered in outpatient infusion centers of the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins (Baltimore) or Sibley Memorial Hospital (Washington DC).

4.1.5 Evaluation of Toxicity and Response

- For dose escalation purposes, the DLT evaluation period is defined as the first 10 weeks of treatment (ie, 6 weeks of CRT plus LC followed by a 4-week post-CRT period.
- Patients are available for DLT evaluation if they have either:
    - Received at least 80% of the planned doses of LC (8 of the planned 10 doses), at least 80% of the planned RT, and at least 60% of the planned TMZ

      OR

- Experienced a DLT in the first 10 weeks of treatment

- Dose finding will be performed using a TITE-BOIN schema as outlined in Section 8.4.9. The window for DLT evaluation and decisions regarding dose advancement or de-escalation will be based on events occurring during the first 10 weeks of the study, though further data regarding safety and tolerability will be collected on every patient continuing infusions through the adjuvant TMZ treatment period.
- Standard contrast magnetic resonance imaging (MRI) will be used for assessment of progression, following the Response Assessment in Neuro-Oncology (RANO) criteria for PD. Patients will have a baseline MRI study followed by CRT, an MRI at the beginning of the adjuvant TMZ phase (4 weeks after completion of CRT) and another MRI every 2 cycles thereafter as per standard of care. More frequent imaging may be clinically necessary per the discretion of the treating physicians.
- MRI scans will be assessed locally by board-certified neuroradiologists who will independently assess tumor size and radiographic changes.
- For the first 2 years after the last dose of LC, patients will be followed for survival and progression every 2 months by telephone call, clinic visit, or medical records. After 2 years have elapsed, patients will be followed every 6 months for survival and progression by telephone call, clinic visit, or medical records.
- For the purposes of analyses, the duration of each arm will last through the 6 weeks of CRT, the 4-week post-CRT period, and the adjuvant cycles received by the patient.

4.1.6 Safety Monitoring

- The study will be overseen by an SRC composed of the Medical Monitor and 2 voting members from the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins. The SRC will convene to review data and discuss next steps at the end of every dose level (once all patients complete the 10-week evaluation period), the data have been cleaned, and the DLT outcomes are determined. In addition, the SRC will meet every 6 months at minimum, and as needed to review AEs. The Principal Investigator and study biostatistician will attend SRC meetings. The SRC will sign off on each dose level before activation of a new dose cohort or expansion of a dose cohort may begin.
- All women of childbearing potential (WOCBP) should be instructed to contact the Investigator immediately if they suspect they might be pregnant (eg, missed or late menstrual period) at any time during study participation. The Investigator must immediately notify the Medical Monitor in the event of a confirmed pregnancy in a patient participating in the study.
- The reason(s) for protocol therapy discontinuation, the reason(s) for study removal, and the corresponding dates must be documented in the Case Report Form (CRF).

- Adverse events will be followed for 30 days after the last dose of LC. Patients removed from the study for unacceptable AEs will be followed until resolution or stabilization of the AE.

4.2 Scientific Rationale for Study Design

Administration of CRT is the standard of care for patients with HGG. In this study, LC will be used in combination with standard therapy.

4.3 Justification for Dose

A recommended starting dose for weekly administration has been established at LC 300 mg/m^2 IV. The maximum dose in the Phase 1b trial was 300 mg/m^2 (Greil et al, 2018).

In an FIH safety and PK study in healthy patients, doses ranging from 1 to 400 mg/m^2 were administered with premedication of diphenhydramine alone.

The systemic safety of LC was good over the dose range between 10 and 320 mg/m^2. In patients dosed at 400 mg/m^2, markers of hemolysis were observed, without clinical symptoms.

5 STUDY POPULATION

Prospective approval of protocol deviations to recruitment and enrollment criteria, also known as protocol waivers or exemptions, is not permitted.

5.1 Inclusion Criteria

Women and members of minority groups will be actively recruited. No exclusion to this study will be based on gender, race, or ethnicity.

Patients are eligible to be included in the study only if all of the following criteria apply:

1. Capable of giving signed informed consent as described in Section 9.1.3 (Appendix 1) that includes compliance with the requirements and restrictions listed in the ICF and in this protocol.
2. ≥ 18 years of age
3. Histologically confirmed HGG (WHO grade III or IV, including GBM, astrocytoma, gliosarcoma, *H3K27M* mutant diffuse midline glioma). Patients with methylated or unmethylated O(6)-methylguanine-DNA methyltransferase (MGMT) promoter are eligible, as are IDH WT and mutant patients as long as the treatment plan is for combined RT/TMZ. The neuropathologic diagnosis of HGG will be made at the respective institution. If any question arises regarding the accuracy of the neuropathologic diagnosis, slides (and pathological blocks, if necessary) will be centrally reviewed.
4. Planning standard therapy with TMZ and RT for 6 weeks
5. Karnofsky Performance Scale (KPS) ≥ 60% (Section 9.6 [Appendix 6])
6. Adequate organ and marrow function defined as:

Hgb	> 9 g/dL
ANC	≥ 1500/µL
Platelet count	≥ 100,000/µL
Total bilirubin	≤ 1.5 * institutional ULN
AST and ALT	≤ 3 * institutional ULN
Creatinine	≤ 1.5 * institutional ULN OR
Estimated glomerular filtration rate (eGFR)	≥ 60 mL/min/1.73 m^2 unless data exist supporting safe use at lower values of renal function, but eGFR must be ≥ 30 mL/min/1.73 m^2

7. Patients with human immunodeficiency virus (HIV) who are on effective antiretroviral therapy are eligible if the viral load was assessed as undetectable within 6 months prior to baseline.
8. Women: WOCBP must agree to use adequate contraception (hormonal or barrier method of birth control or abstinence) prior to study entry and for the duration of study participation.

   Men: must agree to use adequate contraception prior to study entry, for the duration of study participation, and for 4 months after completion of LC administration.

5.2 Exclusion Criteria

Patients are excluded from the study if any of the following criteria apply:

1. Any concurrent cancer diagnosis that is untreated, actively treated, or has undergone any therapy (RT, cytotoxic, targeted, immunotherapeutic, etc) within 2 years of study enrollment, with the exception of squamous or basal cell skin cancer
2. Patient has not recovered from AEs due to prior anticancer therapy (ie, residual toxicities > Grade 1), with the exception of alopecia
3. Receiving any other investigational agent
4. Active infection requiring systemic antibiotics
5. History of allergic reaction to compounds that are chemically or biologically similar to LC (see Section 5.5.1.2 and Section 5.5.1.3)
6. Patient is taking a medication that may potentiate hemolysis
7. Unstable angina or myocardial infarction within the past 6 months
8. Prolonged QTc interval, Bazett formula (QTcB) (> 450 msec for males or > 460 msec for females)
9. Psychiatric illness or social situation that could limit compliance with study requirements
10. Pregnant or breastfeeding

5.3 Screen Failures

Screen failures are defined as patients who consent to participate in the clinical study but do not meet the eligibility criteria. A minimal set of screen failure information is required to ensure transparent reporting of screen failure patients to meet the Consolidated Standards of Reporting Trials (CONSORT) publishing requirements and to respond to queries from regulatory authorities. Minimal information includes demography, screen failure details, eligibility criteria, and any serious adverse event (SAE).

Individuals who do not meet the criteria for participation in this study (screen failure) may be rescreened. A patient who is rescreened is required to sign another ICF.

5.4 Study Intervention

Study intervention is defined as any investigational intervention(s), marketed product(s), placebo, or medical device(s) intended to be administered to a study patient according to the study protocol.

5.5 Study Interventions Administered

Table 5. Regimen Description - Concurrent CRT Period

Agent	Premedications / Precautions	Dose	Route	Schedule	Cycle Length
LC	Dexamethasone 4 mg IV Diphenhydramine 25 mg IV	Per treatment assignment	IV over 3 hr	Weekly: Weeks 1, 2, 3, 4, 5, 6	6 weeks
TMZ	No food 2 hr before and after dosing Antiemetic (eg, ondansetron, prochlorperazine) 30 minutes before dosing Stool softener PRN	75 mg/m^2	PO	Daily during term of RT	
RT		2 Gy	External beam therapy	Monday-Friday	

IV = intravenous; LC = liposomal curcumin; PO = per os (oral); PRN = pro re nata (as needed); RT = radiotherapy; TMZ = temozolomide

Table 6. Regimen Description - Post-CRT Period

Agent	Premedications / Precautions	Dose	Route	Schedule	Cycle Length
LC	Dexamethasone 4 mg IV Diphenhydramine 25 mg IV	Per treatment assignment	IV over 3 hr	Weekly: Weeks 7, 8, 9, 10	4 weeks

IV = intravenous; LC = liposomal curcumin

Table 7. Regimen Description - Adjuvant Period

Agent	Premedications / Precautions	Dose	Route	Schedule	Cycle Length
LC	Dexamethasone 4 mg IV Diphenhydramine 25 mg IV	Per treatment assignment	IV over 3 hr	Weekly: Adjuvant Cycles 1-6: Weeks 1, 2, 3, 4 of each cycle	4 weeks
TMZ	No food 2 hr before and after dosing	150-200 mg/m^2 (Cycles 1-6)	PO	Daily	

Agent	Premedications / Precautions	Dose	Route	Schedule	Cycle Length
	Antiemetic (eg, ondansetron, prochlorperazine) 30 minutes before dosing Stool softener prn				

CRT = chemoradiotherapy; IV = intravenous; LC = liposomal curcumin; PO = per os (oral); PRN = pro re nata (as needed); TMZ = temozolomide

5.5.1 Curcumin

Curcumin is a natural product found in the plant turmeric.

5.5.1.1 General Information

Commercial name: LipoCurc

Chemical name: Diferuloylmethane

Molecular formula: $C_{21}H_{20}O_6$

Molecular weight: 368.38

International Union of Pure and Applied Chemistry (IUPAC) name: (1E,6E)-1,7-bis (4-hydroxy- 3-methoxyphenyl) -1,6- heptadiene-3,5-dione

How supplied: Polymun Scientific GmbH (Vienna, Austria)

5.5.1.2 Potential Toxicity of Liposomal Curcumin

No specifically known drug interactions will exclude patients from enrollment. In early studies, curcumin has been shown to exaggerate effects from *Ginkgo*, hypoglycemic agents, and cholesterol-lowering agents, and patients should be monitored for these metabolic effects or encouraged to reduce or eliminate these medications from their regimen. According to in vitro studies, there is no to minimal inhibition or induction of the common CYP450 drug metabolism enzymes. As part of the enrollment/informed consent process, the patient will be counseled on the risk for interactions with other agents, and what to do if new medications need to be prescribed or if the patient is considering a new over-the-counter medicine or herbal product.

5.5.1.3 Potential Toxicity of Liposomal Curcumin Administered with Temozolomide

This study will investigate the safety and tolerability of LC when co-administered with the alkylating agent TMZ as routinely administered during standard of care CRT and adjuvant chemotherapy regimens in the treatment of newly diagnosed HGG. Currently, there is no human safety study to suggest that there are any risks or benefits to this combination. However, interferences with cytotoxic drugs such as irinotecan, mechlorethamine, doxorubicin, and cyclophosphamide, as well as synergistic effects with 5-fluorouracil may occur. Enhancement or exaggeration of *Ginkgo biloba* effects,

hypoglycemia with diabetes medications, and lipid lowering with cholesterol-lowering drugs may also be observed. Appropriate monitoring of blood sugar and cholesterol parameters should be performed in patients with these comorbid conditions.

CYP450 studies have been conducted and show no tendency for inhibition or induction of the common CYP450 drug metabolism enzymes. It is therefore not expected that LC will have any major drug-drug interactions. No specific restrictions with respect to other drugs metabolized via CYP450 enzymes is necessary.

5.5.1.4 Liposomal Curcumin Dosing Precautions

Because of the potential for allergenic reactions with liposomal drug delivery vehicles, patients will be predosed with 4 mg IV dexamethasone and 25 mg diphenhydramine prior to each infusion. Patients who are already on maintenance dexamethasone of at least 4 mg daily can defer the additional IV premedication of dexamethasone if this medication has been orally administered on the morning prior to their infusions.

Recommended dose adjustments based on symptoms are described in Section 5.10.

5.5.2 Temozolomide

5.5.2.1 General Information

Generic name: Temozolomide

Commercial name: Temodar®

IUPAC name: 3,4-dihydro-3-methyl-4-oxoimidazo[5,1-d]-tetrazine-8 carboxamide

Classification: Antineoplastic agent, alkylating

Molecular formula: $C_6H_6N_6O_2$

Molecular weight: 194.15

Appearance: White to light tan/light pink powder

Melting point: Decomposes at 206ºC

How supplied: Commercially available

5.5.2.2 Mechanism of Action

Temozolomide is not directly active but undergoes rapid non-enzymatic conversion at physiologic pH to the reactive compound 5-(3-methyltriazen-1-yl)- imidazole-4-carboxamide (MTIC). The cytotoxicity of MTIC is thought to be primarily due to alkylation of DNA. Alkylation (methylation) occurs mainly at the O_6 and N_7 positions of guanine.

5.5.2.3 Stability

The molecule is stable at acidic pH (< 5), and labile at pH > 7, hence TMZ can be administered orally. The prodrug, TMZ, is rapidly hydrolyzed to the active MTIC at

neutral and alkaline pH values, with hydrolysis taking place even faster at alkaline pH. The product label recommends storage at 25°C (77°F); excursions permitted to 15°C to 30°C (59°F to 86°F).

5.5.2.4 Half-life

Temozolomide is rapidly eliminated with a mean elimination half-life of 1.8 hr.

5.5.2.5 Packaging, Dispensing, and Storage

TEMODAR capsules are available in strengths of 5, 20, 100, 140, 180, and 250 mg. The capsules contain a white capsule body with a color cap and the colors vary based on the dosage strength. The 5-mg, 20-mg, 100-mg, 140-mg, and 180-mg capsule strengths are available in 5-count and 14-count packages. The 250-mg capsule strength is available in a 5-count package. Labeling of the packages containing the capsules will be done in accordance with the local procedures (as required by law). The hospital pharmacist will deliver the TMZ dosage for a complete cycle to the patient.

5.5.2.6 Known Potential Toxicities of Temozolomide

Known potential toxicities of TMZ are hematological toxicities (leucopenia, lymphopenia, thrombocytopenia, and anemia), renal insufficiency, nausea and vomiting, liver enzyme abnormalities, lethargy, rash, headache, alopecia, constipation, fatigue/malaise, anorexia, hyperglycemia, and diarrhea. Recently, cases of hepatic injury, including fatal hepatic failure, have been observed in patients enrolled in clinical studies utilizing TMZ. In addition, it was noted that liver toxicity may occur several weeks or more after initiation of treatment or after TMZ discontinuation. Refer to the package insert for additional information on AEs observed to date (TEMODAR package insert, 2019).

Rats given TMZ in recent multidose toxicity studies have developed adenocarcinoma of the breast, fibrosarcomas, malignant schwannomas (a variant of fibrosarcoma), keratoacanthomas, and basal cell adenomas. Similar studies conducted in dogs did not reveal any similar findings. The significance of this finding for humans is not known presently.

If liver function tests (alkaline phosphatase, total bilirubin, SGOT, SGPT) are abnormal, the decision to initiate TMZ treatment should carefully consider the benefits and risks for the individual patient. For patients with significant liver function abnormalities, the benefits and risks of continuing treatment should be carefully considered.

Temozolomide is potentially mutagenic and should be handled with appropriate precautions by both staff and patients. Patients with known or suspected hypersensitivity to TMZ should not be treated with TMZ. There are no data available on the effect or management of TMZ overdose. Additional information is available in the TMZ package insert (TEMODAR package insert, 2019).

5.5.3 Temozolomide Dosing Precautions

Nausea Prevention/Treatment: Patients will be instructed to administer TMZ at night before bedtime on an empty stomach. Patients will be prescribed ondansetron at 8 mg to administer 30 minutes prior to chemotherapy ingestion as well as every 8 hr as needed. Patients can additionally be prescribed prochlorperazine at 5-10 mg as needed every 6 hr per Investigator preference.

Constipation Prevention/Treatment: Patients may be prescribed polyethylene glycol 3350 (eg, MiraLAX®) 16 g daily as needed, docusate (50-100 mg every 6 hr as needed), or senna glycoside (20 mg every 12 hr as needed) per Investigator preference.

Recommended dose adjustments based on symptoms are described in Section 5.10.

For patients receiving concurrent TMZ and LC, blood counts will be evaluated weekly. Within -5 days (5 days prior to) the first dose of each 5-day TMZ treatment, the patient must have an ANC ≥ 1500/µL and platelet count ≥ 100,000/µL. On Day 1 of each cycle (within -5 days) all nonhematological Grade 3 or 4 toxicities (except for alopecia, nausea and vomiting, and lymphopenia), that is definitely, probably, or possibly related to TMZ must have resolved (Common Terminology Criteria for Adverse Events [CTCAE] Grade ≤ 1). If toxicity persists, treatment should be delayed by 1 week for up to 3 consecutive weeks. If after 3 weeks of delay all toxicity has still not resolved, any further adjuvant treatment with TMZ should be stopped.

5.5.4 Radiotherapy

No specific prophylaxis is indicated for the RT regimen. The Investigator may use low-dose benzodiazepines if clinically indicated for anxiolytic management if compliance is an issue.

Modalities: Only conformal techniques (3-dimensional conformal radiotherapy [3D CRT] or intensity-modulated radiotherapy [IMRT]) will be allowed in this study. Proton radiotherapy is not allowed. Two-dimensional techniques not employing volumetric target volume definition or 3-dimensional assessments of radiation dose are not allowed. The radiation technology utilized as well as the dosing and target volumes are determined according to current standard of care and described below. In most circumstances IMRT will be optimal as most target volumes are convex or concave, such that there would likely be an advantage in protection of critical structures and brain.

Equipment: All patients must be treated with a linear accelerator with nominal photon energy between 6 to 18 MV. Co-60 is not allowed on this study. Electrons and protons may not be used.

Additional information on treatment planning may be found in Section 9.2 (Appendix 2).

5.5.4.1 Potential Toxicity of Radiotherapy

Side effects of radiation may occur in an increased frequency and severity when receiving LC along with standard RT and TMZ.

The following side effects may be associated with RT for brain cancer:

Likely:

- Tiredness including mental slowing
- Lack of energy
- Redness of soreness of scalp
- Hair loss (temporary or permanent)
- Short-term hearing loss
- Possible increase in brain tumor symptoms such as headaches, seizures, or weakness during treatment
- Edema or selling of the brain that may require steroid medication

Less Likely:

- Nausea
- Vomiting
- Permanent mental slowing
- Permanent hearing loss
- Cataracts
- Behavioral change
- Temporary worsening of existing neurological deficits, such as decreased vision, drowsiness
- Changes in the level of some hormones related to changes to the pituitary gland
- Dry mouth or altered taste

Uncommon but Serious:

- Severe local damage to normal brain tissue, a condition called necrosis (tissue deterioration). Radiation necrosis can mimic recurrent brain tumor and may require surgery for diagnosis and treatment.
- Injury to brain causing permanent neurologic deficits or disability
- Injury to brain causing severe cognitive slowing
- Injury to the eyes with the possibility of blindness

Rare

- Development of other tumors (either benign or malignant)

5.6 Preparation/Handling/Storage/Accountability

1. The Investigator or designee must confirm appropriate temperature conditions for study interventions have been maintained during transit for all study intervention

received and any discrepancies are reported and resolved before use of the study intervention.

2. Only patients enrolled in the study may receive study intervention and only authorized site staff may supply or administer study intervention. All study intervention must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the Investigator and authorized site staff.
3. The Investigator, institution, or the head of the medical institution (where applicable) is responsible for study intervention accountability, reconciliation, and record maintenance (ie, receipt, reconciliation, and final disposition records).
4. Further guidance and information for the final disposition of unused study interventions are provided in the Laboratory Manual.

5.7 Measures to Minimize Bias: Randomization and Blinding

This is an open-label study that is not controlled, randomized, or blinded. Patients with be assigned to a dose level of LC at study entry, based on order of entry and decisions made by the SRC using the TITE-BOIN design described in Section 8.

5.8 Study Intervention Compliance

Patient compliance with TMZ will be assessed at each visit following the weeks during which TMZ is administered in the CRT and adjuvant periods. Compliance will be assessed by direct questioning and by counting returned capsules. Deviations from the prescribed dosage regimen should be recorded in the CRF.

5.9 Concomitant Therapy

Prohibited concomitant therapies are listed in Section 5.9.1. Other than these agents, no specifically known drug interactions will exclude patients from enrollment. In early studies, curcumin has been shown to exaggerate effects from *Ginkgo* spp., hypoglycemic agents, and cholesterol-lowering agents, and patients should be monitored for these metabolic effects or encouraged to reduce or eliminate these medications from their regimen. According to in vitro studies, there is no to minimal inhibition or induction of the common CYP450 drug metabolism enzymes. As part of the enrollment/informed consent procedures, the patient will be counseled on the risk of interactions with other agents, and what to do if new medications need to be prescribed or if the patient is considering a new over-the-counter medicine or herbal product.

Any medication or vaccine (including over-the-counter or prescription medicines, vitamins, and/or herbal supplements) that the patient is receiving at the time of enrollment or receives during the study must be recorded along with:

- Reason for use
- Dates of administration including start and end dates
- Dosage information including dose and frequency

The Medical Monitor should be contacted if there are any questions regarding concomitant or prior therapy.

Patients must abstain from taking curcumin supplements during the study.

5.9.1 Prohibited Therapies

No investigational or commercial agents or therapies may be used during the study to treat the patient's malignancy other than those specified by this protocol.

In addition, the following commonly-used drugs and drug classes are not permitted during the study:

- Levofloxacin
- Penicillins
- Cephalosporins
- Ibuprofen (patients should also be instructed not to take this medication at home)
- Methyldopa
- Quinidine

5.9.2 Rescue Medicine

Patients who exhibit possible infusion reactions despite premedication should be discussed with the Medical Monitor.

5.10 Dose Modification

This protocol allows some alteration from the currently outlined dosing schedule, but the maximum dose of LC will not exceed 400 mg/m^2.

The decision to proceed to the next dose level of (either an increase or a decrease) will be made by the SRC and the Investigator based on safety and tolerability observed in at the patients at the prior dose level, with the dosing decisions indicated in the TITE-BOIN design.

The dosing schedule may also be adjusted to expand a dosing cohort to further evaluate safety and tolerability findings at a given dose level. The study procedures for these additional patient(s)/cohort(s) will be the same as that described for other study patients/cohorts.

If moderate or severe AEs are consistently observed across patients in a cohort or if unacceptable toxicity, reasonably attributable to LC or the combination of LC and TMZ in the opinion of the Investigator are observed, dose escalation may be temporarily halted and no further patients will be dosed until a full safety review of the study by the SRC has taken place. Relevant reporting and discussion with the Medical Monitor, relevant personnel, and the Institutional Review Board (IRB)/Independent Ethics Committee (IEC) will take place before resumption of dosing.

If the same SAE occurs in more than 2 patients in a cohort, dose escalation will be temporarily halted and no further patients will be dosed until a full safety review of the data has taken place. Relevant reporting and discussion with the Medical Monitor, relevant personnel, and the IRB/IEC will take place before resumption of dosing.

6 DISCONTINUATION OF STUDY INTERVENTION AND PATIENT DISCONTINUATION/WITHDRAWAL

6.1 Discontinuation of Study Intervention

Patients who discontinue treatment with LC will continue to be followed for AEs for an additional 30 days after the last dose of LC and the last dose of TMZ, and then for progression-free survival and overall survival as described in Section 4.1.6.

See the Schedule of Assessments (SoA) for data to be collected at the time of discontinuation of study intervention.

Discontinuation of study intervention for abnormal liver function should be considered by the Investigator when a patient meets one of the conditions outlined in the eligibility criteria of if the Investigator believes that it is in best interest of the patient.

If a clinically significant finding is identified (including, but not limited to changes from baseline in QTc interval after enrollment (using the Bazett formula [QTcB]), the Investigator or qualified designee will determine if the patient can continue in the study and if any change in patient management is needed. This review of the ECG printed at the time of collection must be documented. Any new clinically relevant finding should be reported as an AE. Males with QTcB interval > 450 msec and females with QTcB interval > 460 msec should discontinue treatment with LC. Any patient with an increase from baseline in the QTcB interval > 15 msec should discontinue LC.

See the SoA for data to be collected at the time of intervention discontinuation and follow-up and for any further evaluations that need to be completed.

6.1.1 Temporary Discontinuation of Radiation

Administration of LC will continue through a temporary discontinuation of radiation.

6.1.2 Rechallenge

A patient who develops a DLT or AE attributed to LC that requires discontinuation of LC will not be rechallenged with LC.

6.2 Patient Discontinuation/Withdrawal from Study

- A patient may withdraw from the study at any time at their own request, or may be withdrawn at any time at the discretion of the Investigator for safety, behavioral, compliance, or administrative reasons.
- At the time of discontinuing from the study, if possible, an End of Treatment visit should be conducted, as shown in the SoA. The SoA indicates what data is to be collected at the time of study discontinuation and follow-up and any further evaluations that need to be completed. The patient will be permanently discontinued both from the study intervention and from the study at that time.

- If the patient withdraws consent for disclosure of future information, the Sponsor may retain and continue to use any data collected before such a withdrawal of consent.
- If a patient withdraws from the study, they may request destruction of any samples taken and not tested, and the investigator must document this in the site study records.

6.3 Lost to Follow-up

A patient will be considered lost to follow-up if they repeatedly fail to return for scheduled visits and is unable to be contacted by the study site.

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

- The site must attempt to contact the patient and reschedule the missed visit as soon as possible and counsel the patient on the importance of maintaining the assigned visit schedule and ascertain whether or not the patient wishes to and/or should continue in the study.
- Before a patient is deemed lost to follow-up, the Investigator or designee must make every effort to regain contact with the patient (where possible, 3 telephone calls and, if necessary, a certified letter to the patient's last known mailing address or local equivalent methods). These contact attempts should be documented in the patient's medical record.
- Should the patient continue to be unreachable, they will be considered to have withdrawn from the study.

7 STUDY ASSESSMENTS AND PROCEDURES

- Study procedures and their timing are summarized in the SoA. Protocol waivers or exemptions are not allowed.
- Immediate safety concerns should be discussed with the Sponsor immediately upon occurrence or awareness to determine if the patient should continue or discontinue study intervention.
- Adherence to the study design requirements, including those specified in the SoA, is essential and required for study conduct.
- All screening evaluations must be completed and reviewed to confirm that potential patients meet all eligibility criteria. The Investigator will maintain a screening log to record details of all patients screened and to confirm eligibility or record reasons for screening failure, as applicable. A patient who is rescreened is required to sign another ICF.
- Procedures conducted as part of the patient's routine clinical management (eg, blood count) and obtained before signing of the ICF may be utilized for screening or baseline purposes provided the procedures met the protocol-specified criteria and were performed within the timeframe defined in the SoA.

7.1 Safety Assessments

Safety objectives are identification of the MTD/RP2D of LC in combination with RT and TMZ and adjuvant TMZ.

7.1.1 Definition of Evaluable for Safety

All patients will be evaluable for toxicity from the time of their first study treatment.

7.1.2 Assessments

As detailed in Section 7.2, safety assessments will consist of monitoring and recording all AEs and SAEs, the regular monitoring of hematology and blood chemistry, pregnancy testing (in WOCBP), regular measurement of vital signs, and the performance of physical/neurological examinations. ECGs and other cardiac monitoring may be performed as necessary.

Planned time points for all safety assessments are provided in the SoA (Section 1.3).

7.1.3 Primary Safety Endpoints

7.1.3.1 Dose-Limiting Toxicity, Dose Escalation, and Assessment of Primary Endpoints

Dose escalation will proceed within each cohort according to the scheme below (Table 10).

7.1.3.2 Maximum Tolerated Dose and Recommended Phase 2 Dose

Maximum Tolerated Dose: The MTD will be determined using a TITE-BOIN design as described in Section 8.

Recommended Phase 2 Dose: The RP2D will be determined after reviewing the safety data at each dose level.

7.1.4 Secondary Safety Endpoints

7.1.4.1 Physical Examinations

- A complete physical examination will include, at a minimum, assessments of the cardiovascular, respiratory, gastrointestinal, and neurological systems. Height and weight will also be measured and recorded.
- A brief physical examination will include, at a minimum, assessments of the cardiopulmonary and neurological systems. New findings must be reported as AEs.
- Investigators should pay special attention to clinical signs related to previous serious illnesses.

7.1.4.2 Vital Signs

- Oral or tympanic temperature, pulse rate, respiratory rate, and systolic and diastolic blood pressure will be assessed.
- Blood pressure and pulse measurements will be assessed with a completely automated device. Manual techniques will be used only if an automated device is not available.
- Vital signs will be measured in a semisupine position after 5 minutes of rest and will include temperature, systolic and diastolic blood pressure, and pulse.

7.1.4.3 Electrocardiograms

Single 12-lead ECG will be obtained as outlined in the SoA (see Section 1.3) using an ECG machine that automatically calculates the heart rate and measures PR, QRS, QT, and QTc intervals. The Bazett formula will be used to calculate QTc intervals. Males with QTcB interval > 450 msec and females with QTcB interval > 460 msec should discontinue treatment with LC. Any patient with an increase from baseline in the QTcB interval > 15 msec should discontinue LC.

7.1.4.4 Clinical Safety Laboratory Assessments

- Clinical laboratory safety monitoring will occur as per the study calendar in Section 1.3. Laboratory monitoring will be typical of that employed during standard therapy for newly diagnosed malignant gliomas.

  - During weeks 2, 3 and 4 of CRT, a CBC with differential will be performed, including the parameters listed in Table 12. During the post-CRT period when only LC is infused, weekly monitoring with a CBC with differential will continue.
  - CMP will be evaluated on Day 15, Day 22, and then monthly, including the parameters listed in Table 12.
  - Because hemolytic anemia has been described, a baseline peripheral smear and LDH will be performed, and a repeat will be triggered if either of the following are observed:
    - 2-point decrease from baseline in Hgb
    - Hgb < 9 g/dL

- See Section 9.3 (Appendix 3) for the list of clinical laboratory tests to be performed and to the SoA for the timing and frequency.
- The Investigator must review the laboratory report, document this review, and record any clinically relevant changes occurring during the study in the AE section of the CRF. The laboratory reports must be filed with the source documents.
- All laboratory tests with values considered clinically significantly abnormal during participation in the study or within 30 days after the last dose of LC should be repeated until the values return to normal or baseline or are no longer considered clinically significant by the Investigator or Medical Monitor.
- If such values do not return to normal/baseline within a period of time judged reasonable by the Investigator, the etiology should be identified and the Sponsor notified.
- All protocol-required laboratory assessments, as defined in Section 9.3 (Appendix 3), must be conducted in accordance with the laboratory manual and the SoA.
- If laboratory values from nonprotocol-specified laboratory assessments performed at the institution's local laboratory require a change in patient management or are considered clinically significant by the Investigator (eg, SAE or AE or dose modification), then the results must be recorded in the CRF.

7.2 Adverse Events and Serious Adverse Events

The definitions of an AE or SAE can be found in Section 9.4.

AEs will be reported by the patient (or, when appropriate, by a caregiver, surrogate, or the patient's legally authorized representative).

The Investigator and any qualified designees are responsible for detecting, documenting, and recording events that meet the definition of an AE or SAE and remain responsible for following up AEs that are serious, considered related to the study intervention or study

procedures, or that caused the patient to discontinue the study or study interventions (see Section 6).

7.2.1 Reporting Adverse Events

All AEs must be recorded in the electronic data capture system.

7.2.1.1 Adverse Event Description and Grade

The descriptions and grading in the NCI CTCAE version 5.0 will be used in the reporting of AEs. A change in grade will be reported as a separate AE. A copy of the CTCAE v 5.0 may be downloaded from http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

7.2.1.2 Attribution of Adverse Events

The relationship of all AEs to each of the study drugs will be assessed as specified in Table 8.

Table 8. Attribution of Adverse Events

Relationship	Definition
Definite	The AE is clearly related to the study treatment
Probable	The AE is likely related to the study treatment
Possible	The AE may be related to the study treatment
Unlikely	The AE is doubtfully related to the study treatment
Unrelated	The AE is clearly NOT related to the study treatment

AE = adverse event

For all AEs and each treatment (LC, TMZ, and RT), the Investigator will specify the corresponding relationship.

7.2.2 Time Period and Frequency for Collecting Adverse Event Information

All SAEs will be collected from the signing of the ICF until the last follow-up visit at the time points specified in the SoA (Section 1.3).

Adverse events will be collected from the start of treatment and followed for 30 days after the last dose of LC. Patients removed from the study for unacceptable AEs will be followed until resolution or stabilization of the AE (Section 1.3, Section 7.2.4, Section 9.4.3).

Medical occurrences that begin before the start of study intervention but after obtaining informed consent will be recorded on the Medical History/Current Medical Conditions section of the CRF, not the AE section.

All SAEs will be recorded and reported to the Sponsor or designee immediately and under no circumstance should this exceed 24 hr, as indicated in Section 9.4 (Appendix 4). The Investigator will submit any updated SAE data to the Sponsor within 24 hr of it being available.

If the Investigator learns of any SAE, including a death, at any time after a patient has been discharged from the study, and the Investigator considers the event to be definitely, probably, or possibly related to the study intervention or study participation, the Investigator must promptly notify the Sponsor.

7.2.3 Method of Detecting Adverse Events

The method of recording, evaluating, and assessing causality of AEs and SAEs and the procedures for completing and transmitting SAE reports are provided in Section 9.4 (Appendix 4).

Care will be taken not to introduce bias when detecting AEs and/or SAEs. Open-ended and nonleading verbal questioning of the patient is the preferred method to inquire about AE occurrences.

7.2.4 Follow-up of Adverse Events

After the initial AE/SAE report, the Investigator is required to proactively follow each patient at subsequent visits/contacts. All SAEs will be followed until resolution, stabilization, the event is otherwise explained, or the patient is lost to follow-up (as defined in Section 6.3). Further information on follow-up procedures is given in Section 9.4 (Appendix 4).

7.2.5 Regulatory Reporting Requirements for Serious Adverse Events

- Prompt notification by the Investigator to the Sponsor of a SAE is essential so that legal obligations and ethical responsibilities towards the safety of patients and the safety of a study intervention under clinical investigation are met.
- The Sponsor has a legal responsibility to notify both the local regulatory authority and other regulatory agencies about the safety of a study intervention under clinical investigation. The Sponsor will comply with country-specific regulatory requirements relating to safety reporting to the regulatory authority, IRB/IEC, and Investigators.
- Investigator safety reports must be prepared for suspected unexpected serious adverse reactions (SUSARs) according to local regulatory requirements and Sponsor policy and forwarded to Investigators as necessary.
- An Investigator who receives an investigator safety report describing a SAE or other specific safety information (eg, summary or listing of SAEs) from the Sponsor will review and then file it along with the Investigator's Brochure and will notify the IRB/IEC, if appropriate according to local requirements.

7.2.6 Pregnancy

- The effects of LC on the developing human fetus are unknown, but possible teratogenic effects have been observed in animal and in vitro models. For this reason and because LC as well as TMZ used in this trial are known to be

teratogenic, WOCBP and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately. Men treated or enrolled on this protocol must also agree to use adequate contraception prior to the study, for the duration of study participation, and 4 months after completion of LC administration.

- Pregnant women are excluded from this study because the curcuminoid moiety of LC has the potential for teratogenic or abortifacient effects at high doses. Specifically, mouse studies using high-dose curcumin have suggested lower fetal birth weights (Chen et al, 2010; Ganiger et al, 2007), and using pegylated curcumin showed reduced live birth rates in mature females (Murphy et al, 2012). This same study showed hastened onset of puberty in immature female mice, also raising concerns of potential risk for AEs in nursing infants secondary to treatment of the mother with LC, and breastfeeding should therefore be discontinued if the mother is treated with LC. These potential risks may also apply to other agents used in the study, including but not limited to the co-administration with TMZ which is associated with embryo-fetal toxicity.
- Details of all pregnancies in female patients and in female partners of male patients will be collected from the time of consent until 90 days after the last dose of study drug.
- If a pregnancy is reported, the Investigator should inform the Sponsor within 24 hr of learning of the pregnancy and should follow the procedures outlined in Section 9.5 (Appendix 5).
- Abnormal pregnancy outcomes (eg, spontaneous abortion, fetal death, stillbirth, congenital anomalies, ectopic pregnancy) are considered SAEs.

7.2.7 Deaths

For the first 2 years after the last dose of LC, patients will be followed for survival every 2 months by telephone call, clinic visit, or medical records. After 2 years have elapsed, patients will be followed every 6 months for survival by telephone call, clinic visit, or medical records.

7.3 Efficacy Assessments

The efficacy endpoints of this study are OS and PFS.

Patients with measurable enhancing disease will be assessed by the HGG RANO criteria. Patients should be re-evaluated 1 month after the completion of concurrent CRT as well as every 2 cycles (approximately every 8 weeks) with a contrast-enhanced cranial MRI scan. Evidence of PD will be determined as outlined in the RANO criteria below.

7.3.1 Definition of Evaluable for Efficacy

Enrolled patients are newly diagnosed and there are no restrictions on amount of measurable disease from biopsy, subtotal, or gross total resection. These patients will have their disease evaluated according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of Cycle 1 will also be considered evaluable.)

7.3.2 Disease Parameters

Measurable Disease: Bidimensionally, contrast-enhancing, measurable lesions with clearly defined margins by MRI scan, with a minimal size of 1 cm by 1 cm, and visible on 2 axial slices which are at least 5 mm apart with 0-mm skip. Measurement of tumor around a cyst or surgical cavity, if necessary, requires a minimum thickness of 3 mm. If there are > 2 lesions (multifocal) at baseline, the Investigator must choose the largest 2 to be followed before a patient is entered on study. The remaining lesions will be considered nonmeasurable for the purpose of objective response determination. Unless progression is observed, objective response can only be determined when all measurable and nonmeasurable lesions are assessed.

Target Lesions: In multifocal disease, all measurable lesions within the brain should be identified as **target lesions** and recorded and measured at baseline. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

7.3.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed on post-resection imaging.

Magnetic resonance imaging will be used to evaluate lesions. The same technique should be used to characterize each identified and reported lesion at baseline and during follow-up.

The technical specifications of the scanning sequences used should be optimized if performed outside of the Hopkins system. A glioma-specific radiological protocol has been standardized for scanners within the Hopkins system. Lesions should be measured/assessed on the same pulse sequence as was used at baseline. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans.

7.3.4 Use of Criteria from Response Assessment in Neuro-Oncology for Assessment of Progression

Progressive disease is defined by *any* of the following:

a) ≥ 25% increase in sum of the products of perpendicular diameters of enhancing lesions compared to the smallest tumor measurement obtained either at baseline

(if no decrease) or best response, on stable or increasing doses of corticosteroids. (Stable doses of corticosteroids include patients not on corticosteroids.)

b) Significant increase in T2-fluid-attenuated inversion recovery (FLAIR) nonenhancing lesion on stable or increasing doses of corticosteroids compared to baseline scan or best response following initiation of therapy, not due to comorbid events (RT, demyelination, ischemic injury, infection, seizures, postoperative changes, or other treatment effects)

c) Any new lesion

d) Clear clinical deterioration not attributable to other causes apart from the tumor (eg, seizures, medication adverse effects, complications of therapy, cerebrovascular events, infection, etc) or changes in corticosteroid dose. A diagnosis of clinical progression or deterioration may be made at the discretion of the treating physician.

e) Failure to return for evaluation due to death or deteriorating condition

f) Clear progression of nonmeasurable disease

7.3.5 Overall Survival

Overall survival is defined as the duration of time from the start of treatment with LC to the time of death. For the first 2 years after the last dose of LC, patients will be followed for survival every 2 months by telephone call, clinic visit, or medical records. After 2 years have elapsed, patients will be followed every 6 months for survival by telephone call, clinic visit, or medical records.

7.3.6 Progression-free Survival

Progression-free survival is defined as the duration of time from the start of treatment with LC to the time of progression or death, whichever occurs first. For the first 2 years after the last dose of LC, patients will be followed for progression every 2 months by telephone call, clinic visit, or medical records. After 2 years have elapsed, patients will be followed every 6 months for progression by telephone call, clinic visit, or medical records.

7.3.7 Treatment of Overdose

For this study, any dose of LC greater than the assigned dose level for the patient within a 24-hr time period will be considered an overdose.

The Sponsor does not recommend specific treatment for an overdose. There is no antidote to LC. In the event of an overdose, the Investigator or treating physician should:

1. Contact the Medical Monitor immediately.
2. Closely monitor the patient for any AE/SAE and laboratory abnormalities for at least 2 days.

3. Document the quantity of the excess dose as well as the duration of the overdose in the CRF.

Decisions regarding dose interruptions or modifications will be made by the Investigator in consultation with the Medical Monitor based on the clinical evaluation of the patient.

7.4 Pharmacokinetics

Pharmacokinetic parameters are not evaluated in this study.

7.5 Pharmacodynamics

Pharmacodynamic parameters are not evaluated in this study.

7.6 Genetics

Genetics are not evaluated in this study.

7.7 Biomarkers

No specific exploratory or mandatory biomarkers are evaluated in this Phase 1/2 study of LC.

8 STATISTICAL CONSIDERATIONS

8.1 Statistical Hypotheses

There are no formal statistical hypotheses to be tested in this Phase 1/2 study. All statistical analyses will be descriptive in nature. Exact 95% confidence intervals may be generated for select endpoints.

8.2 Sample Size Determination

The study will enroll up to 24 patients in the dose escalation phase. Once the MTD is established, accrual may continue in the dose expansion phase with the objective of studying 6 additional patients at the MTD dose determined in the escalation phase.

8.3 Populations for Analyses

For purposes of analysis, the following populations are defined:

Table 9. Populations for Analysis

Population	Description
Enrolled	All patients who sign the ICF
Evaluable (Escalation Phase)	All escalation patients who receive at least 80% of the planned doses of LC, 80% of planned RT. and 60% of planned TMZ in the first 10 weeks or experience a DLT prior to receiving 10 doses of LC
Efficacy Evaluable (Expansion Phase)	All expansion patients who receive at least 1 dose of LC and have baseline and at least one post-baseline tumor assessment.
Safety	All patients who receive at least 1 dose of LC. Patients will be analyzed according to the dose level they actually receive.

DLT = dose-limiting toxicity; ICF = Informed Consent Form; LC = liposomal curcumin; RT = radiation therapy; TMZ = temozolomide

8.4 Statistical Analyses

Summaries will be presented by dose level and over all dose levels. The summaries will present descriptive statistics (mean, standard deviation, median, minimum, and maximum) for continuous variables and frequencies and percentages for categorical variables.

Exact 95% confidence intervals may be generated for select endpoints. All data will be included in patient listings.

The statistical analysis plan (SAP) will be developed and finalized prior to database lock. The SAP will provide additional details on the statistical analyses, including procedures for accounting for missing data and outliers.

A high-level summary of the planned statistical analyses of the primary and secondary endpoints is presented below. If there are any differences between the analyses described in the protocol and the SAP, the SAP will prevail.

8.4.1 Disposition and Demographics

Summaries and descriptive statistics of demographic and baseline characteristics will be provided. Descriptive statistics for age, gender, race, ethnicity, height, weight, and body mass index will be generated.

Study and treatment completion status will be summarized, with reasons for premature discontinuation of treatment and of the study presented.

A summary of the number of patients in each study population will also be generated.

8.4.2 Baseline Characteristics and Protocol Deviations

Baseline characteristics and number of protocol deviations (any, minor and major) will be summarized overall and by dose group.

8.4.3 Drug Exposure

Drug exposure will be summarized based on the number of doses received for each study treatment (LC, CRT and RT) as well as the total dose received (separately for LC, CRT and RT), the number of cycles of CRT and the duration of treatment for LC, CRT and RT. Dose interruptions and adjustments will also be summarized.

8.4.4 Karnofsky Performance Scale

Scores on the KPS will be summarized at each assessment. Shift tables from baseline to maximum observed KPS score may also be generated.

8.4.5 Concomitant Medications

Concomitant medications will be summarized using the World Health Organization Drug Dictionary (WHODrug). Prior concomitant medications (medications used prior to study enrollment and stopped prior to the first study drug administration) will be summarized separately.

8.4.6 Safety Analyses

All safety analyses will be performed on the Safety Population. Safety analyses will be presented by dose level and overall. Separate safety analyses for the escalation and expansion phases may be generated for patients treated at the MTD for select safety endpoints.

Safety endpoints include DLTs, AEs, changes in laboratory values, ECGs, and changes in vital signs. All AE terms will be coded using MedDRA preferred term and system organ class.

The proportion of patients experiencing at least 1 DLT as well as the number of DLTs observed will be summarized using frequency table techniques; Exact 95% confidence intervals may be generated. Adverse events occurring after the first study intervention will be summarized as follows: all AEs, SAEs, AEs related to study drug, AEs by severity, AEs by grade using CTCAE, and AEs leading to death.

Laboratory and vital sign data will be summarized as continuous variables by dose level and time point. Changes from baseline will be presented along with observed value at each time point. Select laboratory parameters may be graded using CTCAE. Grades for these lab parameters will be summarized in shift tables comparing the baseline grade to the maximum observed post-baseline grade.

8.4.7 Efficacy Analyses

Overall survival will be estimated using the Kaplan-Meier method, with quartile estimates and associated 95% confidence intervals presented. Patients who are alive at the time of analysis will be censored at the last date that they were known to be alive (last study visit if the patient is still on treatment, or last long-term survival assessment where the patient was known to be alive).

Progression-free survival, defined as the time from the day of first study treatment to disease progression or death, whichever occurs first, will be estimated using Kaplan-Meier techniques based on RANO criteria. Patients without evidence of progression will be censored at their last tumor response assessment.

8.4.8 Dose Escalation Rule

The window used for assessing DLTs in this study is 10 weeks. In the TITE-BOIN dose escalation design, the risk of a DLT is assumed to increase over the 10-week DLT assessment period. We operationalize this assumption by breaking up the DLT assessment period into thirds (3.33 weeks) and distribute the risk of DLT within each third. We assign 20% of the overall risk of DLT during the first third, 30% during the middle third, and 50% of the risk during the last third of the 10-week assessment period.

There are 4 dose levels for this study (see Table 4), with the first group of patients starting at Dose Level 1. Dose Level -1 will be utilized if the toxicity profile for Dose Level 1 is shown to be unacceptable. The TITE-BOIN design will be used for dose escalation decisions. Whereas most Phase 1 dose escalation designs are based on occurrence of a DLT, the TITE-BOIN design uses a dose escalation algorithm that incorporates time to experiencing a DLT, thereby accounting for patients who have not completed their DLT assessment period (pending patients).

A TITE-BOIN design requires the level of DLT risk that one can tolerate. For the design below, this risk is set to 30%. In the design one also chooses a target interval of DLT risk; the TITE-BOIN design assigns dose levels based on the highest likelihood of DLT risk being within this range. That is, the dose escalation strategy seeks the dose level with the highest probability of being within this interval given the information already observed. Operationally, we will stop considering any dose (or higher doses) if the data lead to the conclusion that there is 95% probability that this dose is greater than the MTD.

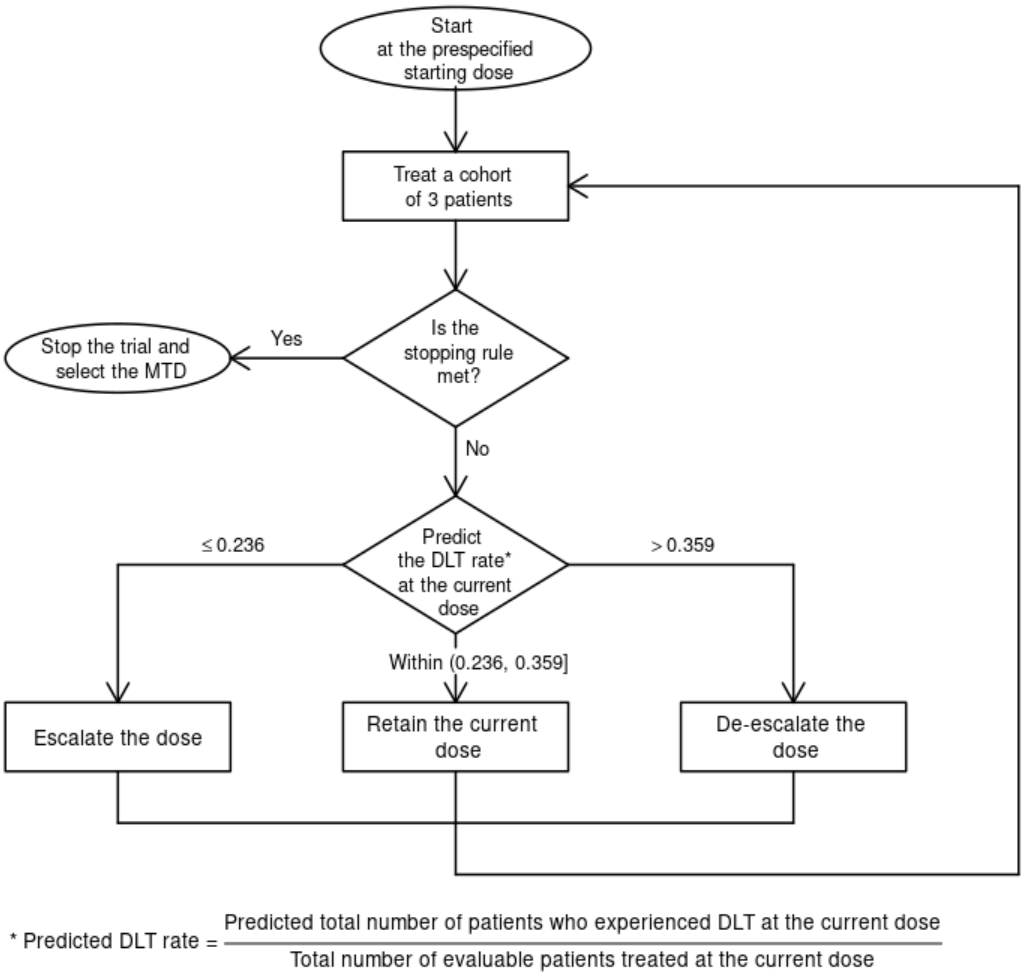
We will use a cohort size of 3 patients for dose escalation decisions based on the TITE-BOIN design. The first cohort will receive 300 mg/m^2 (Dose Level 1) of LC weekly. The proposed target DLT risk is 30%, and the target DLT range is (23.6%, 35.9%). The dose escalation algorithm will drop doses at or above any dose for which the

observed data suggest there is 95% probability that this dose results in a DLT rate above 30%. Patient enrollment to the escalation phase will stop when 12 patients are assigned to the same dose or 24 patients have been enrolled, whichever happens first. Details of TITE-BOIN design are available at the website https://trialdesign.org/one-page-shell.html#TITE-BOIN.

8.4.9 Dose Escalation Algorithm

Figure 2 illustrates the dose escalation scheme based on TITE-BOIN design.

Figure 2. Dose Escalation Algorithm



DLT = dose-limiting toxicity; MTD = maximum tolerated dose

Similar to 3+3 design, in the TITE-BOIN design, when a new patient is ready to enroll in the dose escalation phase, the dose level to be assigned will depend on the outcome of patients who are assigned to the current dose level. For each dose level, the decision whether a new patient should be assigned to the current dose level (stay), the next higher dose level (escalation), or the next lower dose level (de-escalation) is provided in Table

10. The first column is the number of evaluable patients who have been assigned to the current dose level, the second column is the number of patients who experienced DLT at the current dose level, and the third column is the number of patients whose DLT data are pending at the current dose level. In the last column, "Yes & Eliminate" means the next patient will be dosed at the next lower dose level (de-escalation); the current and all higher dose levels will be eliminated from further dosing. Weighted standardized follow-up time (WSTFT) represents a weighted sum of the DLT intervals achieved in patients dosed at the current dose level. Online WSTFT computation is available from the website https://trialdesign.org/one-page-shell.html#TITE-BOIN.

Table 10. TITE-BOIN Design Dose Escalation and De-escalation Rules at Each Dose Level

# Evaluable patients	# DLTs observed	# Pending patients	Escalation	Stay	De-escalation
3	0	<=1	Yes	No	No
3	0	>=2	Suspend	Suspend	Suspend
3	1	0	No	Yes	No
3	1	1	No	WSTFT>0.88	WSTFT<=0.88
3	1	>=2	Suspend	Suspend	Suspend
3	2	<=1	No	No	Yes
3	3	0	No	No	Yes & Eliminate
6	0	<=3	Yes	No	No
6	0	>=4	Suspend	Suspend	Suspend
6	1	<=1	Yes	No	No
6	1	2	WSTFT>=0.6	WSTFT<0.6	No
6	1	3	WSTFT>=1.96	WSTFT<1.96	No
6	1	>=4	Suspend	Suspend	Suspend
6	2	0	No	Yes	No
6	2	1	No	WSTFT>0.73	WSTFT<=0.73
6	2	2	No	WSTFT>1.8	WSTFT<=1.8
6	2	3	No	WSTFT>2.87	WSTFT<=2.87

6	2	>=4	Suspend	Suspend	Suspend
6	3	<=3	No	No	Yes
6	>=4	<=2	No	No	Yes & Eliminate
9	0	<=4	Yes	No	No
9	0	>=5	Suspend	Suspend	Suspend
9	1	<=4	Yes	No	No
9	1	>=5	Suspend	Suspend	Suspend
9	2	0	Yes	No	No
9	2	1	WSTFT>=0.59	WSTFT<0.59	No
9	2	2	WSTFT>=1.65	WSTFT<1.65	No
9	2	3	WSTFT>=2.71	WSTFT<2.71	No
9	2	4	WSTFT>=3.77	WSTFT<3.77	No
9	2	>=5	Suspend	Suspend	Suspend
9	3	0	No	Yes	No
9	3	1	No	WSTFT>0.58	WSTFT<=0.58
9	3	2	No	WSTFT>1.65	WSTFT<=1.65
9	3	3	No	WSTFT>2.72	WSTFT<=2.72
9	3	4	No	WSTFT>3.79	WSTFT<=3.79
9	3	>=5	Suspend	Suspend	Suspend
9	4	<=5	No	No	Yes
9	>=5	<=4	No	No	Yes & Eliminate
12	0	<=6	Yes	No	No
12	0	>=7	Suspend	Suspend	Suspend
12	1	<=6	Yes	No	No
12	1	>=7	Suspend	Suspend	Suspend

12	2	<=3	Yes	No	No
12	2	4	WSTFT>=1.33	WSTFT<1.33	No
12	2	5	WSTFT>=2.72	WSTFT<2.72	No
12	2	6	WSTFT>=4.11	WSTFT<4.11	No
12	2	>=7	Suspend	Suspend	Suspend
12	3	<=6	No	Yes	No
12	3	>=7	Suspend	Suspend	Suspend
12	4	0	No	Yes	No
12	4	1	No	WSTFT>0.43	WSTFT<=0.43
12	4	2	No	WSTFT>1.5	WSTFT<=1.5
12	4	3	No	WSTFT>2.57	WSTFT<=2.57
12	4	4	No	WSTFT>3.65	WSTFT<=3.65
12	4	5	No	WSTFT>4.72	WSTFT<=4.72
12	4	6	No	WSTFT>5.79	WSTFT<=5.79
12	4	>=7	Suspend	Suspend	Suspend
12	5, 6	<=7	No	No	Yes
12	>=7	<=5	No	No	Yes & Eliminate

DLT = dose-limiting toxicity; TITE-BOIN = time-to-event Bayesian optimal interval; WSTFT = weighted standardized follow-up time

8.4.10 Study Design Operating Characteristics

We evaluated the design operating characteristics through simulations for 5 scenarios: 4 situations in which the MTD (DLT risk is 0.3) is at one of the 4 dose levels plus the scenario in which all dose levels have DLT risks > 0.3. The risk of DLT increased across the three thirds of the 10-week assessment period, as described above. The time to toxicity is simulated from a Weibull distribution, with 65% (=30%/2+50%) of the DLTs occurring in the second half of the assessment window, and the patient accrual follows a Poisson process at the rate of 1 patient per 2 months.

We carried out 2000 simulations to estimate the probability of each candidate dose level being selected as the final MTD, the number of patients treated at each dose level, the mean number of patients needed to find the MTD, the probability that the study will stop

early with less than 24 patients, and the duration (in months) to complete the dose escalation study assuming recruitment rate is 1 patient every 2 months (ie, 6 patients per year). The results below show that the design tends to assign patients to the correct dose level in these scenarios.

Operating characteristics based on 2000 simulations under each of 5 scenarios are described in Table 11.

Table 11. Study Design Operating Characteristics Under Simulated Scenarios

	Dose 1	Dose 2	Dose 3	Dose 4	# Patients	% Early Stopping	Duration (months)
Scenario 1							
True DLT rate	0.30	0.47	0.55	0.64			
Selection %	61.6	24.6	2.5	0.1		11.2	39.6
% Pts Treated	49.5	41.0	8.4	1.1	18.2		
Scenario 2							
True DLT rate	0.11	0.30	0.45	0.67			
Selection %	17.6	62.0	19.6	0.6		0.1	43.7
% Pts Treated	23.3	49.8	22.9	4.1	20.7		
Scenario 3							
True DLT rate	0.02	0.13	0.30	0.47			
Selection %	0.6	24.6	58.9	15.8		0.0	45.4
% Pts Treated	4.0	36.9	41.8	17.4	21.6		
Scenario 4							
Selection %	0.6	24.6	58.9	15.8		0.0	45.4
% Pts Treated	4.0	36.9	41.8	17.4	21.6		

Selection %	0.6	24.6	58.9	15.8		0.0	45.4
Scenario 5							
True DLT rate	0.40	0.55	0.70	0.85			
Selection %	61.2	8.0	0.2	0.0		30.7	35.6
% Pts Treated	60.0	35.7	4.1	0.1	15.9		

Pts = patients
Note. "% Early Stopping" refers to early stopping due to excessive DLTs.

8.5 Feasibility Estimation

The feasibility is estimated by the proportion of patients who are evaluable for DLT assessment defined as defined as patients being able to complete 80% of the planned doses of LC, 80% of RT and 60% of TMZ within the first 10 weeks of treatment among all treated patients together with 95% confidence interval. Since this is the first time this treatment combination will be studied, this estimated proportion of evaluable patients will be used as reference for a future Phase 2 study to assess the feasibility of this treatment regime.

8.6 Dose Expansion

An expansion cohort will be enrolled after the MTD has been determined in the escalation portion of the study. A maximum of 6 patients will be enrolled in the expansion cohort, dosed at the MTD. Expansion patients will continue to be monitored for safety to ensure that the DLT risk does not exceed 30%. Specifically, if more than 2 patients in the expansion cohort experience a DLT, an ad hoc SRC meeting may be convened to discuss the acceptability of the MTD.

9 SUPPORTING DOCUMENTATION AND OPERATIONAL CONSIDERATIONS

9.1 Appendix 1: Regulatory, Ethical, and Study Oversight Considerations

9.1.1 Regulatory and Ethical Considerations

- This study will be conducted in accordance with the protocol and with the following:
    - Consensus ethical principles derived from international guidelines including the Declaration of Helsinki and Council for International Organizations of Medical Sciences (CIOMS) International Ethical Guidelines
    - Applicable International Council for Harmonisation (ICH) and Good Clinical Practice (GCP) Guidelines
    - Applicable laws and regulations
- The protocol, protocol amendments, ICF, Investigator Brochure, and other relevant documents (eg, advertisements) must be submitted to an IRB/IEC by the Investigator and reviewed and approved by the IRB/IEC before the study is initiated.
- Any amendments to the protocol will require IRB/IEC approval before implementation of changes made to the study design, except for changes necessary to eliminate an immediate hazard to study patients.
- The Investigator will be responsible for the following:
    - Providing written summaries of the status of the study to the IRB/IEC annually or more frequently in accordance with the requirements, policies, and procedures established by the IRB/IEC
    - Notifying the IRB/IEC of SAEs or other significant safety findings as required by IRB/IEC procedures
    - Providing oversight of the conduct of the study at the site and adherence to requirements of the Code of Federal Regulations (21 CFR), ICH guidelines, the IRB/IEC, European regulation 536/2014 for clinical studies (if applicable), and all other applicable local regulations

9.1.2 Financial Disclosure

Investigators and Subinvestigators will provide the Sponsor with sufficient, accurate financial information as requested to allow the Sponsor to submit complete and accurate financial certification or disclosure statements to the appropriate regulatory authorities. Investigators are responsible for providing information on financial interests during the course of the study and for 1 year after completion of the study.

9.1.3 Informed Consent Process

- The Investigator or their representative will explain the nature of the study to the patient or their legally authorized representative and answer all questions regarding the study.
- Patients must be informed that their participation is voluntary. Patients or their legally authorized representatives will be required to sign a statement of informed consent that meets the requirements of 21 CFR 50, local regulations, ICH guidelines, Health Insurance Portability and Accountability Act (HIPAA) requirements, where applicable, and the IRB/IEC or study center.
- The medical record must include a statement that written informed consent was obtained before the patient was enrolled in the study and the date the written consent was obtained. The authorized person obtaining the informed consent must also sign the ICF.
- Patients must be reconsented to the most current version of the ICF(s) during their participation in the study.
- A copy of the ICF(s) must be provided to the patient or the patient's legally authorized representative.

A patient who is rescreened is required to sign another ICF.

9.1.4 Data Protection

- Patients will be assigned a unique identifier by the Sponsor. Any patient records or datasets that are transferred to the Sponsor will contain the identifier only; patient names or any information which would make the patient identifiable will not be transferred.
- The patient must be informed that their personal study-related data will be used by the Sponsor in accordance with local data protection law. The level of disclosure must also be explained to the patient.
- The patient must be informed that their medical records may be examined by Clinical Quality Assurance auditors or other authorized personnel appointed by the Sponsor, by appropriate IRB/IEC members, and by inspectors from regulatory authorities.

9.1.5 Safety Review Committee

The study will be overseen by an SRC composed of 3 voting members (the Medical Monitor and two additional members). The SRC will convene to review data and discuss next steps at the end of every dose level (once all patients complete the 10-week evaluation period), when the data have been cleaned, and the DLT outcomes are determined. In addition, the SRC will meet every 6 months at minimum, and as needed to review AEs. The Principal Investigator and study biostatistician will attend SRC

meetings. The SRC will sign off on each dose level before activation of a new dose cohort or expansion of a dose cohort may begin.

9.1.6 Dissemination of Clinical Study Data

This study will be posted on clinicaltrials.gov. Publication policies are described in Section 9.1.11.

9.1.7 Data Quality Assurance

- All patient data relating to the study will be recorded on printed or electronic CRF (eCRF) unless transmitted to the Sponsor or designee electronically (eg, laboratory data). The Investigator is responsible for verifying that data entries are accurate and correct by physically or electronically signing the CRF.
- The Investigator must maintain accurate documentation (source data) that supports the information entered in the CRF.
- The Investigator must permit study-related monitoring, audits, IRB/IEC review, and regulatory agency inspections and provide direct access to source data documents.
- Monitoring details describing strategy (eg, risk-based initiatives in operations and quality such as Risk Management and Mitigation Strategies and Analytical Risk-Based Monitoring), methods, responsibilities and requirements, including handling of noncompliance issues and monitoring techniques (central, remote, or on-site monitoring) are provided in the Monitoring Plan.
- The Sponsor or designee is responsible for the data management of this study including quality checking of the data.
- The Sponsor assumes accountability for actions delegated to other individuals (eg, contract research organizations).
- Study monitors will perform ongoing source data verification to confirm that data entered into the CRF by authorized site personnel are accurate, complete, and verifiable from source documents; that the safety and rights of patients are being protected; and that the study is being conducted in accordance with the currently approved protocol and any other study agreements, ICH GCP, and all applicable regulatory requirements.

9.1.8 Source Documents

- Source documents provide evidence for the existence of the patient and substantiate the integrity of the data collected. Source documents are filed at the Investigator's site.
- Data reported on the CRF or entered in the eCRF that are transcribed from source documents must be consistent with the source documents or the discrepancies must be explained. The Investigator may need to request previous medical records

or transfer records, depending on the study. Also, current medical records must be available.

- The definition of what constitutes source data can be found in the Monitoring Plan.

9.1.9 Quality Assurance

Adherence to Protocol Therapy: Screening/baseline source documentation will be submitted stored locally and securely in the study database. As a quality assurance measure for the treatment delivered on this protocol, primary patient records may be reviewed. The records to be examined will be selected retrospectively and at random; complete records must therefore be maintained on each patient treated on the protocol. These records should include primary documentation (eg, laboratory report slips, imaging reports, pathology reports, physician notes, etc), which confirm that:

- The patient met each eligibility criterion.
- Signed informed consent was obtained prior to treatment.
- Treatment was given according to protocol (dated notes about doses given; any reasons for any dose modifications).
- Toxicity was assessed according to protocol (laboratory report slips, etc).
- Response was assessed according to protocol (MRI scan, lab reports, dated notes on measurements and clinical assessment, as appropriate).

9.1.10 Study and Site Closure

The Sponsor reserves the right to close the study site or terminate the study at any time for any reason at the sole discretion of the Sponsor. Study sites will be closed upon study completion. A study site is considered closed when all required documents and study supplies have been collected and a study-site closure visit has been performed.

The Investigator may initiate study-site closure at any time, provided there is reasonable cause and sufficient notice is given in advance of the intended termination.

Reasons for the early closure of a study site by the Sponsor or Investigator may include but are not limited to:

- Failure of the Investigator to comply with the protocol, the requirements of the IRB/IEC or local health authorities, the Sponsor's procedures, or GCP guidelines
- Inadequate recruitment of patients by the Investigator
- Discontinuation of further study intervention development

9.1.11 Publication Policy

- The results of this study may be published or presented at scientific meetings. If this is foreseen, the Investigator agrees to submit all manuscripts or abstracts to

the Sponsor before submission. This allows the Sponsor to protect proprietary information and to provide comments.

- The Sponsor will comply with the requirements for publication of study results. In accordance with standard editorial and ethical practice, the Sponsor will generally support publication of multicenter studies only in their entirety and not as individual site data. In this case, a coordinating Investigator will be designated by mutual agreement.
- Authorship will be determined by mutual agreement and in line with International Committee of Medical Journal Editors (ICMJE) authorship requirements.

9.2 Appendix 2: Radiotherapy Treatment Considerations and Planning

9.2.1 Treatment Volumes

The goal of the treatment planning process is to deliver a uniform dose to a planning target volume (PTV) which includes all known tumor plus a specified margin. The volume of normal brain outside the PTV receiving > 95% of the prescription dose should be minimized. For treatment planning, the targets are defined below.

9.2.1.1 Gross Tumor Volumes (GTV)

GTV1 (for initial phase of RT): The GTV1 is the T1 enhancing and nonenhancing tumor volume (T2 or FLAIR) as visualized on the baseline MRI scan. In cases when another MRI is performed for protocol eligibility, for RT planning, or for clinical reasons, the target identified on the more recent MRI should be used for treatment planning.

GTV2 (for conedown phase of RT): The GTV2 is the T1 enhancing tumor volume as visualized on the postoperative Day 0/1 MI scan. This would include the resection cavity in patients undergoing resection. In cases when another MRI is performed for protocol eligibility, for radiation treatment planning, or for clinical reasons, the target identified on the more recent MRI should be used for treatment planning.

9.2.1.2 Clinical Target Volume (CTV)

The CTV is the GTV plus a margin of 5 mm in all directions. However, the CTV must not extend outside the brain and should be limited by the edge of brain as areas outside the brain are rarely affected by tumor. Care must be taken when extending it inferiorly to minimize the amount of normal cervical spinal cord included. Margin may be reduced at true anatomic boundaries (eg, bone, nonviolated tentorium, ventricle, etc). In uncommon cases where an MRI cannot be utilized for treatment planning due to a pacemaker or other factor, an additional 1-cm margin rather than 5-mm margin should be added to define the CTV1 and CTV2 to the MRI-based definitions below.

CTV1: The CTV1 is the GTV1 plus a margin of 5 mm in all directions, with consideration of the factors described above.

CTV2: The CTV2 is the GTV2 plus a margin of 5 mm in all directions, with consideration of the factors described above.

9.2.1.3 Planning Target Volumes (PTV)

The PTV is the CTV plus a margin in all directions to account for daily setup variation and patient movement, but not beam penumbra or build-up. The margin should not exceed 5 mm and will commonly be 3-5 mm depending upon immobilization device and frequency of image guidance. Individual institutions are encouraged to perform studies defining the appropriate PTV margin based upon institutional immobilization and localization procedures.

As the PTV is not structure limited, it may extend into and below the skull. There is no need to adjust dosimetry of the treatment plan to ensure full target dose to the skin and bone itself as the tumor will not be present there, regardless of positioning uncertainty included with the PTV margins (ie, there is no need to use bolus to increase dose outside the intracranial space).

PTV1: The PTV1 is the CTV1 plus a margin all directions to account for daily setup variation and patient movement (typically 3-5 mm). Adjust for skin avoidance as described above.

PTV2: PTV2 is the CTV2 plus a margin all directions to account for daily setup variation and patient movement (typically 3-5 mm). Adjust for skin avoidance as described above.

9.2.2 Target Dose

The total dose to the prescription point will be 6000 cGy in 30 daily fractions of 200 cGy each. PTV1 will receive 4600 cGy in 23 fractions. PTV2 will receive an additional 1400 cGy in 7 fractions (total 6000 cGy in 30 fractions).

9.2.3 Prescription Point

For 3D treatment planning, the prescription point is at or near the isocenter. The goal of the treatment plan is to encompass at least 97% of the PTV within the 95% isodose surface. IMRT plans may be prescribed to an isodose line provided that dose uniformity guidelines are met. It is recognized that these goals may not be achieved at the discretion of the treatment team if necessary to protect critical tissues.

9.2.4 Dose Definition

The absorbed dose is specified as cGy to muscle.

9.2.5 Tissue Heterogeneity

Calculations shall take into account the effect of tissue heterogeneities.

9.2.6 Dose Uniformity

For conformal planning techniques (3D CRT and IMRT), the 99% PTV shall be encompassed within the 95% isodose surface and no more than 10% of the PTV should receive more than 105% of the prescription dose, as evaluated by dose volume histogram.

9.2.7 Time and Dose Considerations

Fractionation: Patients will receive 1 treatment per day, 5 days per week. All fields will be treated each day. At least 2 fractions must be given during the first week of treatment.

9.2.8 Treatment Technique

Any 3D treatment technique which delivers the appropriate dose to the PTV is permitted. Coplanar and noncoplanar techniques are both allowed, but attention should focus on use of multiple nonoverlapping treatment fields according to optimal standard of care which will also generally reduce skin dose. A treatment planning computed tomography (CT) scan is required. The MRI-defined volumes should be superimposed onto the treatment planning CT.

9.2.9 Simulation

Simulation will be performed using a CT simulator. As the intent is treatment with the tumor-treating field (TTF) array applicator in place, simulation with a standard Aquaplast mask placed over the applicator and the head shaved. At the time of simulation, an additional simulation mask preparation and scan will be performed without the transducer arrays in place. The purpose is to ensure prompt transition to an optimal RT plan with an appropriately immobilization mask should treatment be required to continue without the TTF array in place.

9.2.10 Patient Position and Immobilization

Immobilization devices such as Thermoplastic mask should be used for all patients.

9.2.11 Field Shaping

Field shaping can be done with blocks or multileaf collimation.

9.2.12 Isocenter Verification

The equivalent of orthogonal (anteroposterior and lateral) digitally reconstructed radiographs (DRRs) and corresponding orthogonal weekly portal images (film or electronic) are required. Alternatively, image guidance with kilovoltage imaging and setup DRRs or cone beam CT is acceptable.

9.3 Appendix 3: Clinical Laboratory Tests

- The tests detailed in Table 12 will be performed.
- Protocol-specific requirements for inclusion or exclusion of patients are detailed in Section 5 of the protocol.
- Additional tests may be performed at any time during the study as determined necessary by the Investigator or required by local regulations.
- Pregnancy testing

Table 12. Protocol-Required Safety Laboratory Assessments

Laboratory Assessments	Parameters
Hematology	Hgb Hematocrit RBC count RBC indices: MCV MCH % reticulocytes WBC count with differential: Neutrophils Lymphocytes Monocytes Eosinophils Basophils Platelet count
Clinical Chemistry	Sodium Potassium Calcium Total and direct bilirubin BUN Creatinine Glucose (may be performed without fasting) Total Protein Alkaline phosphatase AST /SGOT ALT / SGPT LDH Phosphorus
Routine Urinalysis	Specific gravity Dipstick: pH Glucose Protein Blood Ketones Bilirubin Urobilinogen Nitrite Leukocyte esterase Microscopic examination (if blood or protein is abnormal)
Other Screening Tests	Urine human chorionic gonadotropin (hCG) pregnancy test (as needed for WOCBP)

ALT = alanine aminotransferase; AST = aspartate aminotransferase; BUN = blood urea nitrogen; hCG = human chorionic gonadotropin; LDH = lactate dehydrogenase; MCV = mean corpuscular volume; MCH = mean corpuscular hemoglobin; RBC = red blood cell; SGOT = serum glutamic-oxaloacetic transaminase; SGPT = serum glutamic-pyruvic transaminase; WBC = white blood count; WOCBP = women of childbearing potential)

Investigators must document their review of each laboratory safety report.

9.4 Appendix 4: Adverse Events: Definitions and Procedures for Recording, Evaluating, Follow-up, and Reporting

9.4.1 Definition of Adverse Event

Table 13. Definition of Adverse Event

AE Definition
• An AE is any untoward medical occurrence in a patient or clinical study patient, temporally associated with the use of study intervention, whether or not considered related to the study intervention. • NOTE: An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease (new or exacerbated) temporally associated with the use of study intervention.

Table 14. Events Meeting the Adverse Event Definition

Events Meeting the AE Definition
• Any abnormal laboratory test results (hematology, clinical chemistry, or urinalysis) or other safety assessments (eg, ECG, radiological scans, vital signs measurements), including those that worsen from baseline, considered clinically significant in the medical and scientific judgment of the Investigator (ie, not related to progression of underlying disease). • Exacerbation of a chronic or intermittent pre-existing condition including either an increase in frequency and/or intensity of the condition. • New conditions detected or diagnosed after study intervention administration even though it may have been present before the start of the study. • Signs, symptoms, or the clinical sequelae of a suspected drug-drug interaction. • Signs, symptoms, or the clinical sequelae of a suspected overdose of either study intervention or a concomitant medication. Overdose per se will not be reported as an AE/SAE unless it is an intentional overdose taken with possible suicidal/self-harming intent. Such overdoses should be reported regardless of sequelae. • "Lack of efficacy" or "failure of expected pharmacological action" per se will not be reported as an AE or SAE. Such instances will be captured in the efficacy assessments. However, the signs, symptoms, and/or clinical sequelae resulting from lack of efficacy will be reported as AE or SAE if they fulfill the definition of an AE or SAE. • The signs, symptoms, and/or clinical sequelae resulting from lack of efficacy will be reported as AE or SAE if they fulfill the definition of an AE or SAE. Also, "lack of efficacy" or "failure of expected pharmacological action" also constitutes an AE or SAE.

9.4.2 Definition of Serious Adverse Event

If an event is not an AE per definition above, then it cannot be an SAE even if serious conditions are met (eg, hospitalization for signs/symptoms of the disease under study, death due to progression of disease).

Table 15. Definition of Serious Adverse Event

A SAE is defined as any untoward medical occurrence that, at any dose:
a. Results in death
b. Is life-threatening The term 'life-threatening' in the definition of 'serious' refers to an event in which the patient was at risk of death at the time of the event. It does not refer to an event, which hypothetically might have caused death, if it were more severe.
c. Requires inpatient hospitalization or prolongation of existing hospitalization In general, hospitalization signifies that the patient has been detained (usually involving at least an overnight stay) at the hospital or emergency ward for observation and/or treatment that would not have been appropriate in the physician's office or outpatient setting. Complications that occur during hospitalization are AEs. If a complication prolongs hospitalization or fulfills any other serious criteria, the event is serious. When in doubt as to whether "hospitalization" occurred or was necessary, the AE should be considered serious. Hospitalization for elective treatment of a pre-existing condition that did not worsen from baseline is not considered an AE.
d. Results in persistent disability/incapacity The term disability means a substantial disruption of a person's ability to conduct normal life functions. This definition is not intended to include experiences of relatively minor medical significance such as uncomplicated headache, nausea, vomiting, diarrhea, influenza, and accidental trauma (eg, sprained ankle) which may interfere with or prevent everyday life functions but do not constitute a substantial disruption.
e. Is a congenital anomaly/birth defect
f. Other situations: Medical or scientific judgment should be exercised in deciding whether SAE reporting is appropriate in other situations such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require medical or surgical intervention to prevent one of the other outcomes listed in the above definition. These events should usually be considered serious. Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse. Examples of such events include invasive or malignant cancers, intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias or convulsions that do not result in hospitalization, or development of drug dependency or drug abuse.

9.4.3 Recording and Follow-Up of Adverse Events

Table 16. Recording and Follow-Up of Adverse Events

AE and SAE Recording
• When an AE/SAE occurs, it is the responsibility of the Investigator to review all documentation (eg, hospital progress notes, laboratory reports, and diagnostics reports) related to the event. • The Investigator will then record all relevant AE/SAE information in the CRF. • It is **not** acceptable for the Investigator to send photocopies of the patient's medical records to the Sponsor in lieu of completion of the /AE/SAE CRF page. • The Investigator will attempt to establish a diagnosis of the event based on signs, symptoms, and/or other clinical information. Whenever possible, the diagnosis (not the individual signs/symptoms) will be documented as the AE/SAE.
Assessment of Intensity
The Investigator will make an assessment of intensity for each AE and SAE reported during the study and assign it to 1 of the following categories: • Mild: An event that is easily tolerated by the patient, causing minimal discomfort and not interfering with everyday activities. • Moderate: An event that causes sufficient discomfort and interferes with normal everyday activities. • Severe: An event that prevents normal everyday activities. An AE that is assessed as severe should not be confused with a SAE. Severe is a category utilized for rating the intensity of an event; and both AEs and SAEs can be assessed as severe. An event is defined as 'serious' when it meets at least 1 of the predefined outcomes as described in the definition of an SAE, NOT when it is rated as severe. Other measures to evaluate AEs and SAEs may be used (eg, CTCAE).

Table 17. Assessment of Causality

Assessment of Causality
• The Investigator is obligated to assess the relationship between study intervention and each occurrence of each AE/SAE. • A "reasonable possibility" of a relationship conveys that there are facts, evidence, and/or arguments to suggest a causal relationship, rather than a relationship cannot be ruled out. • The Investigator will use clinical judgment to determine the relationship. • Alternative causes, such as underlying disease(s), concomitant therapy, and other risk factors, as well as the temporal relationship of the event to study intervention administration will be considered and investigated. • The Investigator will also consult the Investigator's Brochure and/or Product Information, for marketed products, in their assessment. • For each AE/SAE, the Investigator **must** document in the medical notes that they have reviewed the AE/SAE and has provided an assessment of causality.

- There may be situations in which an SAE has occurred and the Investigator has minimal information to include in the initial report to the Sponsor. However, **it is very important that the Investigator always make an assessment of causality for every event before the initial transmission of the SAE data to the Sponsor.**
- The Investigator may change their opinion of causality in light of follow-up information and send a SAE follow-up report with the updated causality assessment.
- The causality assessment is one of the criteria used when determining regulatory reporting requirements.

Table 18. Follow-Up of Adverse Events

Follow-Up of AEs and SAEs
• The Investigator is obligated to perform or arrange for the conduct of supplemental measurements and/or evaluations as medically indicated or as requested by the Sponsor to elucidate the nature and/or causality of the AE or SAE as fully as possible. This may include additional laboratory tests or investigations, histopathological examinations, or consultation with other health care professionals. • If a patient dies during participation in the study or during a recognized follow-up period, if available, the Investigator will provide the Sponsor with a copy of any postmortem findings including histopathology. • New or updated information will be recorded in the originally completed CRF. • The Investigator will submit any updated SAE data to the Sponsor within 24 hr of receipt of the information.

9.4.4 Reporting of Serious Adverse Events

Table 19. Recording of Serious Adverse Events

SAE Reporting to Sponsor via an Electronic Data Collection Tool
• The primary mechanism for reporting an SAE to the Sponsor will be the electronic data collection tool. • The site will enter the SAE data into the electronic system as soon as it becomes available. • After the study is completed at a given site, the electronic data collection tool will be taken off-line to prevent the entry of new data or changes to existing data. • Any AE that meets a protocol-defined criterion as an SAE must be submitted within 24 hours of site awareness on an SAE form to safety@c3-research.com.

9.5 Appendix 5: Contraceptive Guidance and Collection of Pregnancy Information

The effects of LC on the developing human fetus are unknown, but possible teratogenic effects have been observed in animal and in vitro models. For this reason and because LC agents as well as other therapeutic agents (TMZ) used in this trial are known to be teratogenic, WOCBP and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while

she or her partner is participating in this study, she should inform here treating physician immediately. Men treated or enrolled in this study must also agree to use adequate contraception prior to the study, for the duration of study participation, and for 4 months after completion of LC administration.

Pregnant women are excluded from this study because the curcuminoid moiety of LC has the potential for teratogenic or abortifacient effects at high doses. Mouse studies using high-dose curcumin have suggested lower fetal birth weights (Chen et al, 2010; Ganiger et al, 2007), and pegylated curcumin is associated with reduced live birth rates in mature females (Murphy et al, 2012). The same study showed hastened onset of puberty in immature female mice, raising concerns for AEs in nursing infants of mothers treated with LC. Breastfeeding should be discontinued if a mother is being treated with LC. These potential risks also apply to other agents used in the study, including but not limited to the co-administration of TMZ, which is associated with embryo-fetal toxicity.

Women of Childbearing Potential

A woman is considered fertile following menarche and until becoming postmenopausal unless permanently sterile (see below).

If fertility is unclear (eg, amenorrhea in adolescents or athletes) and a menstrual cycle cannot be confirmed before first dose of study intervention, additional evaluation should be considered.

Women in the following categories are not considered WOCBP:

1. Premenarchal
2. Premenopausal female with 1 of the following:
    - Documented hysterectomy
    - Documented bilateral salpingectomy
    - Documented bilateral oophorectomy

For individuals with permanent infertility due to an alternate medical cause other than the above, (eg, mullerian agenesis, androgen insensitivity), Investigator discretion should be applied to determining study entry.

Note: Documentation can come from the site personnel's: review of the patient's medical records, medical examination, or medical history interview.

3. Postmenopausal female
    - A postmenopausal state is defined as no menses for 12 months without an alternative medical cause.
        - Females on hormonal replacement therapy (HRT) and whose menopausal status is in doubt will be required to use one of the nonestrogen hormonal highly effective contraception methods if they wish to continue their HRT

during the study. Otherwise, they must discontinue HRT to allow confirmation of postmenopausal status before study enrollment.

Collection of Pregnancy Information

Male patients with partners who become pregnant

- The Investigator will attempt to collect pregnancy information on any male patient's female partner who becomes pregnant while the male patient is in this study. This applies only to male patients who receive study intervention.
- After obtaining the necessary signed informed consent from the pregnant female partner directly, the Investigator will record pregnancy information on the appropriate form and submit it to the Sponsor within 24 hr of learning of the partner's pregnancy. The female partner will also be followed to determine the outcome of the pregnancy. Information on the status of the mother and child will be forwarded to the Sponsor. Generally, the follow-up will be no longer than 6 to 8 weeks following the estimated delivery date. Any termination of the pregnancy will be reported regardless of fetal status (presence or absence of anomalies) or indication for the procedure.

Female Patients who become pregnant

- The Investigator will collect pregnancy information on any female patient who becomes pregnant while participating in this study. Information will be recorded on the appropriate form and submitted to the Sponsor within 24 hr of learning of a patient's pregnancy.
- The patient will be followed to determine the outcome of the pregnancy. The Investigator will collect follow-up information on the patient and the neonate and the information will be forwarded to the Sponsor. Generally, follow-up will not be required for longer than 6 to 8 weeks beyond the estimated delivery date. Any termination of pregnancy will be reported, regardless of fetal status (presence or absence of anomalies) or indication for the procedure.
- While pregnancy itself is not considered to be an AE or SAE, any pregnancy complication or elective termination of a pregnancy will be reported as an AE or SAE. A spontaneous abortion is always considered to be an SAE and will be reported as such. Any poststudy, pregnancy-related SAE considered reasonably related to the study intervention by the Investigator will be reported to the Sponsor as described in Section 7.2.5. While the Investigator is not obligated to actively seek this information in former study patients, they may learn of an SAE through spontaneous reporting.
- Any female patient who becomes pregnant while participating in the study must discontinue infusions of LC. Modification of standard CRT should be undertaken as per standard clinical practice; the Investigator should consult the Medical Monitor.

9.6 Appendix 6: Karnofsky Performance Scale

Table 20. Karnofsky Performance Scale

Percent	Description
100	Normal, no complaints, no evidence of disease
90	Able to carry on normal activity; minor signs or symptoms of disease
80	Normal activity with effort; some signs or symptoms of disease
70	Cares for self; unable to carry on normal activity or to do active work
60	Requires occasional assistance, but is able to care for most of his/her needs
50	Requires considerable assistance and frequent medical care
40	Disabled, requires special care and assistance
30	Severely disabled, hospitalization indicated. Death not imminent
20	Very sick, hospitalization indicated. Death not imminent
10	Moribund, fatal processes progressing rapidly
0	Dead

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