Abbreviated Title: Ph I/II TMZ+TG02 astrocytoma

CC Protocol #: 17C0009 F

NCT Number: NCT02942264

Version Date: 06/19/2020

Title: Phase I Trial of Zotiraciclib (TG02) Plus Dose-Dense or Metronomic Temozolomide Followed by Randomized Phase II Trial of Zotiraciclib (TG02) plus Temozolomide versus Temozolomide alone in Adults with Recurrent Anaplastic Astrocytoma and Glioblastoma

NCI Principal Investigator: Jing Wu, M.D., PhD
NOB, CCR, NCI
37 Convent Dr. Rm 1142A
Bethesda, MD 20892
Tel: 240-760-6036
Email: jing.wu3@nih.gov

Investigational Agents:

Drug Name:	Zotiraciclib (TG02)
IND Number:	132001
Sponsor:	CCR
Manufacturer:	Adastra Pharmaceuticals, Inc.

Commercial Agents: Temozolomide

Coordinating Center

Institution: National Cancer Institute, Bethesda, MD
Overall Study PI: Jing Wu, MD, PhD
NOB, CCR, NCI
37 Convent Dr. Rm 1142A
Bethesda, MD 20892
Phone: 240-760-6036
E-mail: jing.wu3@nih.gov

PRECIS

Background:

- Zotiraciclib (TG02) is a pyrimidine-based multi-kinase inhibitor that has been shown to have inhibitory effects on CDKs, Janus Kinase 2 (JAK2) and Fm-like tyrosine kinase 3 (Flt3). It is orally administered and penetrates blood brain barrier (BBB). There is clinical experience in using Zotiraciclib (TG02) as both a single agent and in combination with other chemotherapy agents for cancer treatment.
- Temozolomide (TMZ) is an oral alkylating agent that has proven efficacy in anaplastic glioma and glioblastoma. It was approved by the U.S. Food and Drug Administration (FDA) to treat anaplastic astrocytoma and glioblastoma in adults. Both a dose-dense (dd) schedule, 7 days on and 7 days off and a metronomic (mn) daily dosing schedule have been used to treat recurrent high-grade gliomas.
- Our preclinical data have demonstrated that Zotiraciclib (TG02) down-regulates CDK9 activity and its target proteins, such as anti-apoptotic protein Mcl-1, XIAP and survivin. A treatment with Zotiraciclib (TG02) and TMZ has synergistic anti-glioma effects in a variety of glioma models with different genetic background. This serves as the basis for this proposed clinical trial.

Objectives:

Phase I:

- To determine the maximum tolerated dose (MTD) of Zotiraciclib (TG02) plus TMZ using both the dd and mn TMZ schedules in adult patients with recurrent anaplastic astrocytoma or glioblastoma/gliosarcoma.

Phase II:

- To determine the efficacy of Zotiraciclib (TG02) plus TMZ versus TMZ alone in patients with recurrent WHO grade III or IV astrocytoma as determined by progression free survival.

Eligibility:

- Documented pathology diagnosis of anaplastic astrocytoma [WHO grade III], or glioblastoma/gliosarcoma [WHO grade IV] with recurrent disease. If the pathology diagnosis is anaplastic glioma or anaplastic oligoastrocytoma, evidence of either intact 1p/19q chromosomes or molecular features suggesting astrocytic tumor must be present. (including, but not limited to *ATRX* and/or *TP*53 mutation)
- No prior use of bevacizumab as a treatment for brain tumor.
- No more than two prior relapses for Phase I and no more than one prior relapse for Phase II.
- Patients must have recurrent disease, either histologically proven or with imaging suggestive of recurrent disease
- Tumor tissues available for review to confirm the histologic diagnosis.
- Tumor tissue blocks available for molecular profiling analysis.

Design:

Phase I:

- This portion of the study is conducted in two stages: The MTD finding and cohort extension. Two treatment arms and several dose levels are planned.
- In the MTD finding part, TMZ with two alternate schedules (dd and mn) in combination with Zotiraciclib (TG02) will be administered.
- A cohort extension of both arms will be performed at each MTD and the treatment arm with a better progression free survival at 4 months (PFS4) will be selected for the combination treatment arm for Phase II.
- Pharmacokinetic, pharmacogenetic studies and neutrophil analysis will be performed during the cohort extension of both arms.
- A maximum of 72 patients will be enrolled to this component for the trial.

Phase II:

- Patients will be randomized between two competing treatment arms: ("winner" of dd *vs* mn) TMZ + Zotiraciclib (TG02) versus dd/mn TMZ alone using a Bayesian clinical trial design. The dosage for the combination arm will be derived from the MTD determined in the Phase I component of the study.
- The treatment schedule will be identical to that described above in the phase I component, with each cycle comprising 28 days.
- Patients will continue treatment until tumor progression or unacceptable toxicity occurs.
- At progression, patients randomized to the control arm (Temozolomide [TMZ] alone) will be offered the opportunity to continue TMZ and additional treatment with Zotiraciclib (TG02).

TABLE OF CONTENTS

1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objectives

1.1.1.1 Phase I

To determine the maximum tolerated dose (MTD) of Zotiraciclib (TG02) plus TMZ using both the dd and mn TMZ schedules in adult patients with recurrent anaplastic astrocytoma or glioblastoma/gliosarcoma.

1.1.1.2 Phase II

To determine the efficacy of Zotiraciclib (TG02) plus TMZ *versus* TMZ alone in patients with recurrent WHO grade III or IV astrocytoma as determined by progression free survival (PFS).

1.1.2 Secondary Objectives

1.1.2.1 To select the treatment regimen with better PFS4 between Zotiraciclib (TG02) plus dd TMZ or mn TMZ at each of the MTDs following cohort expansion.

1.1.2.2 To determine the objective response rate, PFS at 6 months (PFS6) and overall survival (OS) in the treatment arms.

1.1.2.3 To longitudinally evaluate patient reported outcome measures using self-reported symptom severity and interference with daily activities using the MDASI-BT

1.1.3 Exploratory Objectives

1.1.3.1 To obtain tumor tissues from initial occurrence and /or recurrence to detect T*P53*, *PTEN*, *IDH* mutation, *MYC* amplification status. The level of anti-apoptotic protein, such as Mcl-1 and XIAP, and caspase 3, cleaved PARP, c-Myc, CDK9 expression will be tested, if tissues are available at the time of recurrence.

1.1.3.2 To evaluate the imaging features by magnetic resonance imaging, including perfusion, before and during the treatment every two months.

1.1.3.3 To obtain blood samples after Zotiraciclib (TG02) dose on cycle 1, day -3 along with PK sample collection to analyze the effect of Zotiraciclib (TG02) on neutrophil count and functions.

1.1.3.4 To perform pharmacokinetic and pharmacogenomic studies of Zotiraciclib (TG02) once the MTD is determined in each cohort.

1.1.3.5 To explore the feasibility of Patient-Reported Outcomes Version of the Common Terminology Criteria for Adverse Events (PRO-CTCAE) in brain tumor patients receiving investigational cancer treatments.

1.2 BACKGROUND AND RATIONALE

1.2.1 Disease background

High grade astrocytomas include glioblastoma (GBM) [WHO grade IV] and anaplastic astrocytoma (AA), [WHO grade III]. Both are highly malignant and belong to gliomas. Gliomas

represent 80% of malignant CNS tumors. GBM accounts for 54% of gliomas and AA accounts for 7%.[1]

Histologically, GBM displays a high mitotic index, cellular pleomorphism, and extensive vascular proliferation or necrosis. They are characterized by their intrinsic heterogeneity, high morbidity and mortality. Despite aggressive multimodality treatment approach in surgery, radiation therapy and cytotoxic chemotherapies, the prognosis of patients with GBM remains poor with a median survival rate of less than 18 months.[2] Recurrent GBM has an even more dismal prognosis with a median overall survival of approximately 25 weeks. [3]

Anaplastic astrocytoma (AA) is classified by WHO as a grade III glioma, demonstrating a less aggressive behavior when comparing to GBM. AA frequently transforms to GBM. The treatment of recurrent AA is unsatisfying. Chemotherapy and re-operation for recurrent AA is of modest benefit and only bring short responses. Wong et al reported that a progression free survival rate at 6 months is 31% and the median progression free survival is only 13 weeks based on eight phase II clinical trials that included 150 patients with recurrent AA.[3] In the pivotal clinical trial that led to the accelerated FDA approval in 1999 for TMZ as the treatment of recurrent AA, the PFS6 and median overall survival were reported as 46% and 13.6 months respectively.[4]

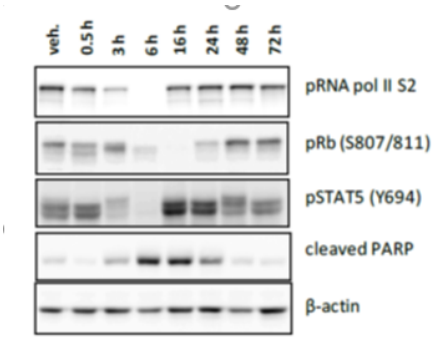
Most normal cells can activate the apoptotic pathways in response to DNA damage or abnormal proliferation. In contrast, tumor cells in AA developed mechanisms to increase cell proliferation and decreasing apoptosis. *TP53* is one of the notably known genes that are implicated in apoptotic pathways of high grade astrocytoma. The *TP53* gene that maps to chromosome 17p is frequently inactivated in astrocytoma and is found in one third of AAs.[5] P53 protein plays multiple important roles in a number of cellular processes including cell cycle arrest, response to DNA damage and apoptosis. As expected, the signaling pathways in gliomas including *TP53* inactivation are most commonly noted in secondary GBM, which evolves from AA, in contrast to the primary glioblastoma, which are featured by genetic alteration, including *EGFR* amplification and chromosome 10 loss with *PTEN* mutation.[6, 7]

1.2.2 Zotiraciclib (TG02) in human cancers

1.2.2.1 Mechanisms of action of Zotiraciclib (TG02)

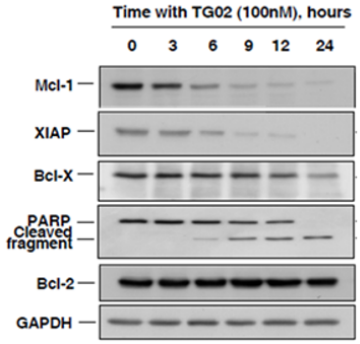
Zotiraciclib (TG02) is a pyrimidine-based multi-kinase inhibitor that has been shown to have inhibitory effects on cyclin-dependent kinases (CDKs), Janus kinase 2 (JAK2) and Fm-like tyrosine kinase 3 (Flt3).[8] It most potently inhibits CDK1, 2, 3, 5 and 9 with an IC_{50} of 3-9 nM and IC_{50} of 37nM for CDK 7. The IC_{50} for JAK2 is 19 nM. It is also effective against Flt3 and its drug resistant mutant with an IC_{50} of 19-21 nM.

Figure 1: Target kinase inhibition by Zotiraciclib (TG02) following oral dosing at 60mg/kg in nude mice bearing MCV-11 AML xenografts.



Among all mechanism of action, CDK9 inhibition is a one of the most appealing features of Zotiraciclib (TG02). CDK9 is a serine/threonine kinase that forms the catalytic core of the positive transcription elongation factor [9-11] and it is critical for stimulating transcription elongation through RNA polymerase II.[12] To assess target engagement by Zotiraciclib (TG02) in tumor tissues, tumor lysate were analyzed after dosing MV4-11 AML xenograft-bearing mice with a single dose of 60mg/kg Zotiraciclib (TG02). Level of RNA-polymerase II phosphorylation at serine 2 was used as a readout for CDK9 inhibition. The inhibition was observed starting from 0.5 hours and reached to a maximum at 6 hours as demonstrated in Figure **1** (Investigator's Brochure, Version 6). As a consequence of inhibiting CDK9 function, the short-lived proteins will be depleted due to their heightened requirement to be regularly refreshed.[13] Two such short-lived proteins that play an important role in cancer cell survival mechanisms are Myeloid Leukemia 1 (Mcl-1), a B-cell Lymphoma 2 (Bcl-2) family member and x-linked inhibitor of apoptosis (XIAP), an anti-apoptotic protein.[14] Both Mcl-1 and XIAP were found to be depleted following a treatment with TG02 in hematologic malignancy cell lines, including myeloma cell line, as demonstrated in Figure **2** (Investigator's Brochure, Version 6).

Figure 2: Zotiraciclib (TG02) depletes anti-apoptotic proteins of the Bcl-2 and AP families in myeloma cell line.



Since it was discovered in 1992, Mcl-1 has been shown to play an integral role in anti-apoptotic signaling pathways. Mcl-1 is highly expressed in a variety of human cancer cells, including CNS cancers. [15] Its overexpression is one of the survival mechanisms that allow cancer cells to survive and to further develop resistance to various apoptotic-inducing therapies. To underscore this point, in high grade gliomas, Mcl-1 was found to be the dominant anti-apoptotic Bcl-2-related protein.[16]

XIAP expression has been noted in human glioma cell lines and apoptosis was blocked after XIAP gene transfer,[17] suggesting that XIAP is involved in apoptosis signaling in human gliomas.

CDK9 activity is regulated though activators, such as c-Myc [18] and NFkB [19], both are heavily involved in pathogenesis of malignant glioma.[20, 21] In addition to their roles in transcription, CDKs can regulate other cellular process that were considered as the core function of cell cycle control mechanisms.[22] While not involved in cell cycle progression, CDK9 and its cyclin partner T1 have a role in cell differentiation.[23] CDK9 was also found to be crucial in MYC-driven tumors. Pharmacological or shRNA-mediated CDK9 inhibition led to anti-tumor effects that correlated with *MYC* expression level, suggesting CDK9 inhibition likely is a therapeutic approach for treating *MYC*-overexpression tumors.[24]

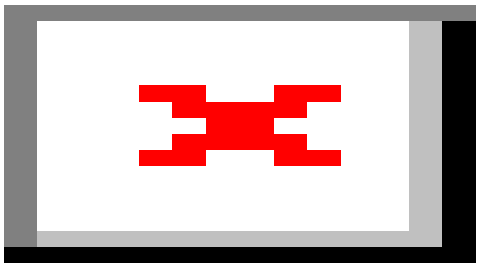
In vitro studies have demonstrated that Zotiraciclib (TG02) depletes survival proteins, including Mcl-1 and XIAP, leading to apoptosis in human tumor cells.[25] An oral dosing at 30-60mg/kg of Zotiraciclib (TG02) in mice AML xenografts demonstrated an inhibition of several target kinases, suggesting that the agent inhibited CDK9, CDK2, JAK2 and FLT3.[8]

1.2.2.2 Preclinical and clinical experience of Zotiraciclib (TG02)

Preclinical experiences

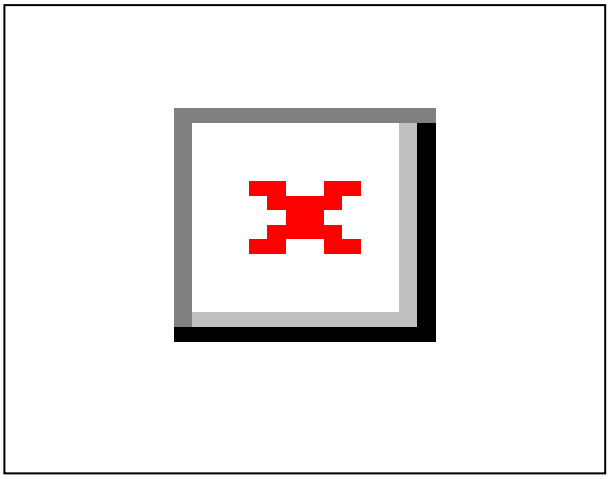
Extensive preclinical experiments have been performed to demonstrate the characteristics of Zotiraciclib (TG02). A Good Laboratory Practice (GLP) safety pharmacology study was conducted in 12 beagle dogs (6 females and 6 male) to evaluate the cardiovascular and respiratory effects of Zotiraciclib (TG02) after a single oral administration at 30mg/kg and 60 mg/kg. A dose-related duration of emesis was observed. One female dog received Zotiraciclib (TG02) at 30mg/kg died due to circulatory failure secondary to diarrhea and dehydration caused by severe intestinal damage. Postmortem pathology exam demonstrated marked atrophy and hemorrhage of thymus in this individual dog. The surface of both small and large intestines was found to be covered by white mucosa layer. The histologic exam showed epithelial necrosis on the mucosa surface and in the lumen of crypts accompanied with marked hyperemia. All the other 11 dogs survived, and no changes were noted in skin and fur, eyes and mucous membranes, respiratory system, circulatory system, autonomic system and CNS. There were no Zotiraciclib (TG02) effects on cardiac function in dogs. The individual values for blood pressure, respiratory rate, body temperature and glucose levels were within physiological limits during the study in all groups. Neither dose- nor sex-dependent variations were observed. Serum chemistry examination performed during the study showed dose dependent escalation in LDH, AST, ALT and CK in interval 2 hours and 6 hours after the administration of Zotiraciclib (TG02) with a trend to recovery of LDH and AST at 24 and 48 hours after Zotiraciclib (TG02) treatment.

Table 1: Pharmacokinetic parameters of Zotiraciclib (TG02) in mice, rats and dogs



Pharmacokinetics (PK) analyses were conducted in female nude mice, male Wistar rats and male beagle dogs. Comparison of single intravenous (I.V.) and oral doses showed variable bioavailability following oral dosing. All three species showed a rapid absorption following oral dosing with T_{max} ranging from 10 minutes to 60 minutes. The PK parameters of Zotiraciclib (TG02) in these 3 species are summarized in Table **1** (Investigator's Brochure, Version 6). The plasma concentration versus time profile of Zotiraciclib (TG02) in female nude mice following a single dose of 5mg/kg intravenously and a single oral dose of 75mg/kg is demonstrated in Figure **3** (Investigator's Brochure, Version 6).

Figure 3: Plasma concentrations vs. time profile of Zotiraciclib (TG02) in female nude mice following a single IV dose of 5mg/kg and a single oral dose of 75 mg/kg (n=3)



The PK assessment following a single oral administration under fed and in unfed conditions in mice suggested no significant food-drug interaction. The percentage of plasma protein biding of Zotiraciclib (TG02) was measured by an equilibrium dialysis of Zotiraciclib (TG02) (1000ng/ml) spiked into human, dog, and mouse plasma against phosphate-buffered saline (PBS) at 37^{o}C for 4 hours. Results suggested that the percentage of plasma-bound Zotiraciclib (TG02) was 99.97% ± 0.01% in human and similar in all species studied. Limited tissue distribution of Zotiraciclib (TG02) was assessed in female nude mice following a single oral dosing at 75mg/kg. The mean concentration of Zotiraciclib (TG02) in plasma and tissues were demonstrated in Figure **4** (Investigator's Brochure, Version 6).

Figure 4: Mean Plasma and tissue concentrations of Zotiraciclib (TG02) determined at different time points following a single oral administration of 75mg/kg to nude mice (n=3)

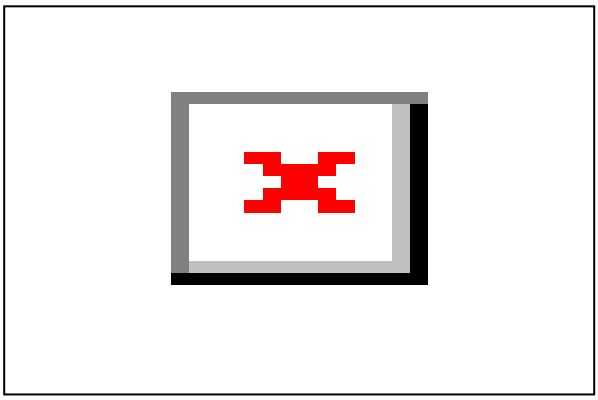
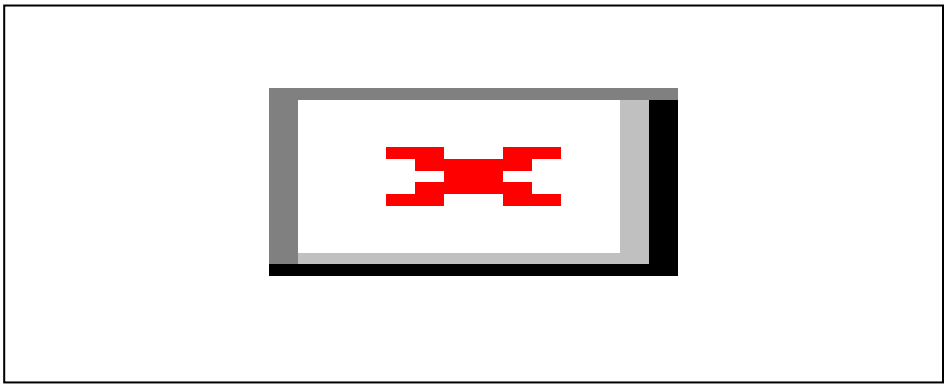


Table 2: Mean Pharmaceutical Parameters of Zotiraciclib (TG02) Following Oral Dosing at 30 mg/kg and 60mg/kg in Nude Mice Bearing MV4-11 AML Xenografts



Zotiraciclib (TG02) preferentially distributed to liver, lungs and kidney followed by heart and brain. The PK of Zotiraciclib (TG02) in plasma and tumor tissues in mice was also determined following a signal dosing of nude mice bearing subcutaneous xenografts of FLT-mutant AML MV4-11 at 30mg/kg and 60mg/kg. The PK parameters that are summarized in Table 2 (Investigator's Brochure, Version 6) suggested that the greater peak and aggregate drug exposure in the tumor than in the blood in this animal model. The tissue distribution after single dose of Zotiraciclib (TG02) in mice demonstrate that the AUC ratio of brain/plasma is 2.4, suggesting good blood brain barrier penetration, which is highly relevant to the potential use of Zotiraciclib (TG02) in brain tumor treatment (Table 3, data from Adastra Pharmaceutical). Figure 5 (Investigator's Brochure, Version 6) shows the mean plasma and tissue concentration profiles over time. The levels of Zotiraciclib (TG02) in plasma and tissue exceeded the biochemical IC_{50} on the kinases CDK1, 2, and 9 (<10 nM) and FLT3 and JAK2 (20 nM to 40 nM); and exceeded the cellular IC_{50} on primary AML blasts (40nM to 50nM) for at least 24 hours at both doses. In

animals receiving an oral dose of 60mg/kg Zotiraciclib (TG02), intratumoral Zotiraciclib (TG02) concentrations peaked at 200-fold above these levels (~8 µM) and remained 10-fold over (~0.4 µM) after 24 hours. By contrast, Zotiraciclib (TG02) was cleared relatively rapidly from plasma, with blood levels dropping below the threshold for cellular activity within 3 to 4 hours after a 30mg/kg dose and 13 to 14 hours after a 60 mg/kg dose.

Table 3: Limited Tissue distribution of Zotiraciclib (TG02) after a single PO administration at 75mg/kg

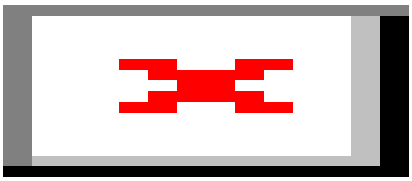
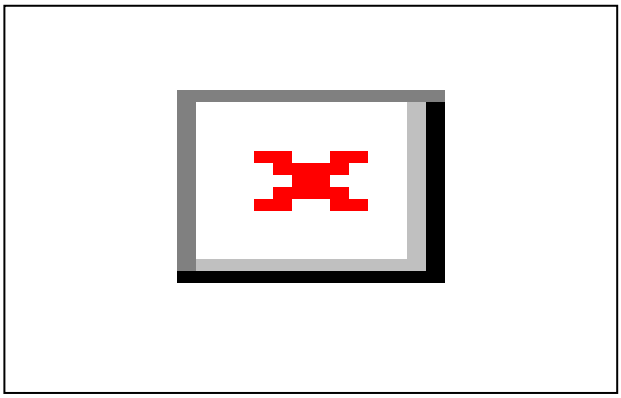
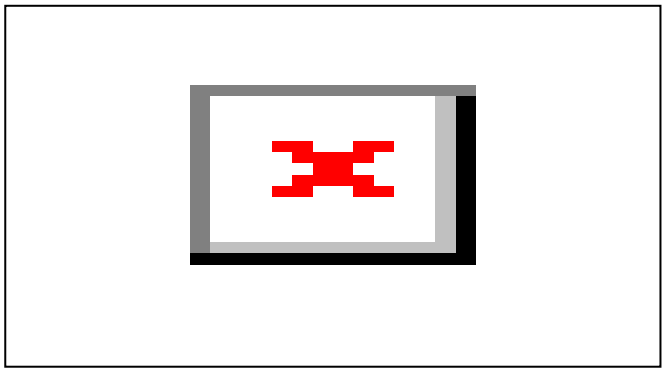


Figure 5: Mean Plasma and Tumor Concentrations of Zotiraciclib (TG02) Following Oral Dosing at 30mg/kg and 60mg/kg in Nude Mice Bearing MV4-11 AML Xenografts



The *in vitro* metabolic $T_{1/2}$ of Zotiraciclib (TG02) in human liver microsomes (LM; $T_{1/2}$ = 48 minutes), indicates involvement of Phase 1 metabolism. Species differences for *in vitro* metabolic $T_{1/2}$ were noted as demonstrated in Figure 6 (Investigator's Brochure, Version 6). The compound appears to be more stable in human and dog than in mouse and rat LMs. The major metabolites observed in all species were M1, N-de-methylation, and M2, resulting from oxidation of Zotiraciclib (TG02).

Figure 6: In-vitro Microsomal Stability of Zotiraciclib (TG02) In Liver Microsomes



To investigate the role of CYP450 isozymes in the metabolism of Zotiraciclib (TG02), CYP3A4, 2D6, 1A2, 2C9, and 2C19 Bactosomes (recombinant isoform expression systems) were incubated with 5 µM Zotiraciclib (TG02) at 37°C for 45 minutes in the presence of nicotinamide adenine dinucleotide phosphate-oxidase. In this assay system, the t1/2 of Zotiraciclib (TG02) was approximately 30 minutes for CYP1A2 and CYP3A4 Bactosomes but >45 minutes for the other isoforms. These results indicate that Zotiraciclib (TG02) is primarily metabolized by CYP1A2 and CYP3A4. The potential for Zotiraciclib (TG02) to inhibit CYP450 isozymes was assessed using pooled human LM. The capacity of Zotiraciclib (TG02) (0.05, 0.25, 0.5, 2.5, 5, and 25 µM) to inhibit various CYP450 isozymes was investigated by measuring the inhibition potential (IC50 and inhibition constant [Ki]) on prototype reactions using specific substrates. Zotiraciclib (TG02) inhibited the catalytic activity of CYP2D6 (IC50 = 0.95 µM ± 0.16 µM), but not the other isozymes tested. The potential of Zotiraciclib (TG02) to induce human CYP3A4 and CYP1A2 isozymes was investigated in cultured human hepatocytes from a single male human donor. Results suggested that Zotiraciclib (TG02) is a weak inducer of CYP1A2 in human hepatocytes when compared with positive control omeprazole. For CYP3A4, Zotiraciclib (TG02) caused no significant induction at the concentration range tested. Trans-epithelial transport of Zotiraciclib (TG02) through Caco-2 monolayers in comparison with 2 control compounds indicates that Zotiraciclib (TG02) has high intestinal absorption. The efflux ratio value (~1) for Zotiraciclib (TG02) suggests no involvement of an active P-glycoprotein mediated transport mechanism.

The toxicological profile of Zotiraciclib (TG02) was evaluated in mice and dogs following single and multiple oral doses for up to 28 days followed by a 28-day non-treatment recovery period. Genotoxicity (Ames test, *in vitro* chromosomal aberration assay, and *in vivo* mouse micronucleus test) was also conducted to provide an overall assessment of Zotiraciclib (TG02). The mean toxicokinetic parameters for Zotiraciclib (TG02) following a single oral administration to mice is summarized in Table 4 (Investigator's Brochure, Version 6) and those following 28 days of daily oral administration is summarized in Table 5 (Investigator's Brochure, version 6).

Table 4: Mean Toxicokinetic Parameters for Zotiraciclib (TG02) Following a Single Oral Administration to Mice

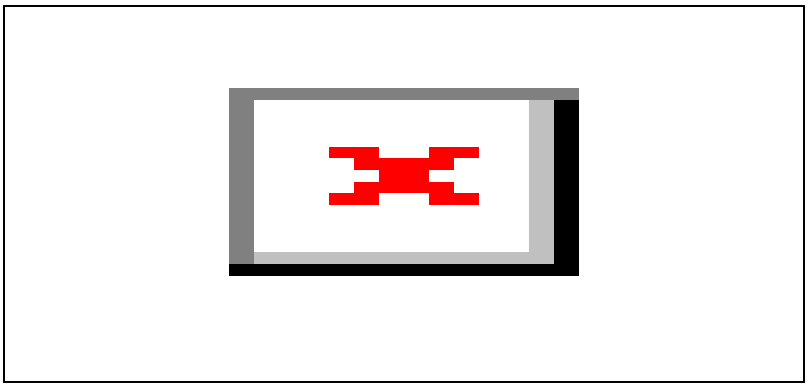
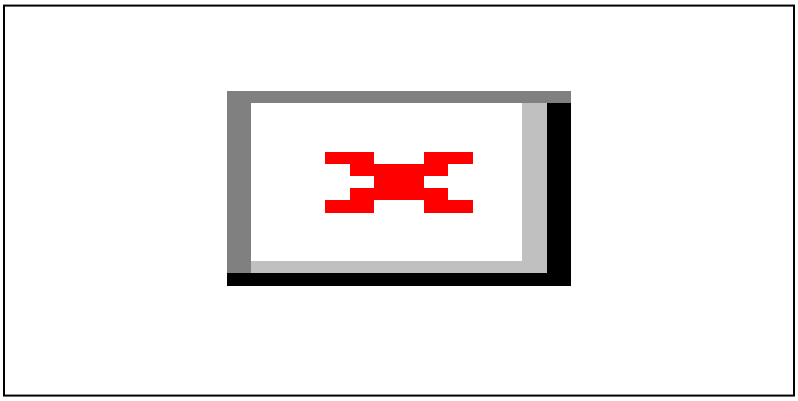


Table 5: Mean Toxicokinetic Parameters for Zotiraciclib (TG02) Following 28 Days of Oral Administration to Mice.



In the GLP acute oral toxicity study in mice, groups of 10 mice/sex were administered 0 (vehicle), 25 mg/kg, 50 m/kg, or 100 mg/kg Zotiraciclib (TG02). Cohorts of mice were humanely terminated on Days 3 and 15 and given a full postmortem evaluation. In addition, a subgroup of mice was designated for neurobehavioral assessment through the use of a functional observational battery (FOB), and another subgroup of mice was designated for a full TK profile on Day 1. All animals survived until scheduled termination. Rough coat was observed on Day 3 in two males received 100 mg/kg and one male received 50 mg/kg. Body weight decreases were observed in the males and females in 100 mg/kg group on Days 2 and 3 and were considered the result of the test article administration. There were no changes in food consumption considered the result of the test article administration. The FOB suggests that a single oral dose of 50 mg/kg or 100 mg/kg Zotiraciclib (TG02) may induce acute increases in aggression and/or emotionality, consistent with minor increases in specific measures of spontaneous locomotor activity (e.g., rearing). Test article-related hematology changes included decreased reticulocytes on Day 3 in the 25 mg/kg males, and in both sexes of the 50 mg/kg and 100 mg/kg groups, which correlated with bone marrow atrophy/necrosis. Total WBC and differential WBC counts decreased in the 50 mg/kg males only and in both sexes of the 100 mg/kg groups. These were the result of test

article administration and were consistent with thymic atrophy observed in the 100 mg/kg animals. Test article-related increases in platelet counts were noted in the 100 mg/kg females. There were no obvious changes in the serum chemistry parameters for any of the groups attributed to Zotiraciclib (TG02) citrate. Histopathologic changes due to Zotiraciclib (TG02) citrate were observed in the thymus, spleen, and bone marrow of mice examined on Day 3, and in the spleen of mice examined on Day 15. Zotiraciclib (TG02)-related atrophy was present in the thymus of all 100mg/kg mice examined at Day 3. Due to the thymic atrophy on Day 3, thymic weight parameters were reduced in both sexes of the 100 mg/kg group and in the 50 mg/kg males. Thymic atrophy was not evident in recovery mice examined on Day 15. Bone marrow atrophy and/or necrosis were present on Day 3 in most 100 mg/kg mice and in some 25 mg/kg and 50 mg/kg females and one (1) 50 mg/kg male. Bone marrow atrophy was not evident in recovery mice examined on Day 15. Hematopoiesis was reduced in the spleen of 100 mg/kg and 50 mg/kg mice and occasional 25 mg/kg females examined on Day 3 and was responsible for reduced splenic weight parameters in the 100 mg/kg males. Splenic hematopoiesis was not apparent in recovery group mice examined on Day 15. Based on the results of the single-dose study of Zotiraciclib (TG02) citrate in mice, a no observed adverse effect level was not determined. Test article-related findings on Day 3 included rough coat (50 mg/kg and 100 mg/kg); body weight decreases (100 mg/kg); decreased reticulocytes (25 mg/kg [males only], 50 mg/kg, and 100 mg/kg); decreased WBC counts (50 mg/kg [males only] and 100 mg/kg); increased platelet counts (100 mg/kg [females only]); and thymic, spleen, and bone marrow changes in organ weights and histopathology (25, 50, and 100 mg/kg). By Day 15, recovery was evident or complete for each of the parameters affected.

Multiple dose toxicity was evaluated by a GLP oral 28-day toxicity study was conducted in CD-1 mice to determine potential toxicity and TK of Zotiraciclib (TG02) citrate when administered by a daily oral gavage for up to 28 consecutive days and to monitor the persistence or reversibility of any adverse effects after a 28-day recovery period. Ten mice/sex/group were assigned to 1 of 4 dose groups at target dosage levels of 0 (control article), 10 mg/kg, 20 mg/kg, and 40 mg/kg Zotiraciclib (TG02). Endpoints used to evaluate the potential toxicity of Zotiraciclib (TG02) citrate were survival, clinical observations, body weights, food consumption, clinical pathology, necropsy, organ weights, and selected histopathology. Mice administered 10 mg/kg/day Zotiraciclib (TG02) did not display any adverse clinical signs or statistically significant differences in mean body weight, food consumption, clinical pathology or organ weight values when compared to respective control values. Preliminary data revealed significant decreases in mean body weight and food consumption values in the 40 mg/kg males and females with a noted decrease in survival in this group. Based on the inability to field recovery animals at 40 mg/kg (exceeded the MTD), animals within the core and recovery groups were reassigned so that recovery could also be assessed at the mid-dose level, 20 mg/kg. Mice administered 40 mg/kg displayed adverse clinical observations suggestive of a general debilitation in their health status; whereas only rough hair coat was noted in some mice administered 20 mg/kg.

Administration of 40 mg/kg Zotiraciclib (TG02) was related to decreases in reticulocyte counts, red blood cell (RBC) counts, hemoglobin, and hematocrit in males and females, and decreases in total WBC, lymphocyte, neutrophil, and monocyte counts in the males on Day 29. Mice administered 20 mg/kg Zotiraciclib (TG02) displayed decreases in total WBC, lymphocyte,

neutrophil, and monocyte counts on Day 29. On Day 57, the hematology results of the 20 mg/kg mice of both sexes and the 40 mg/kg females were similar to controls.

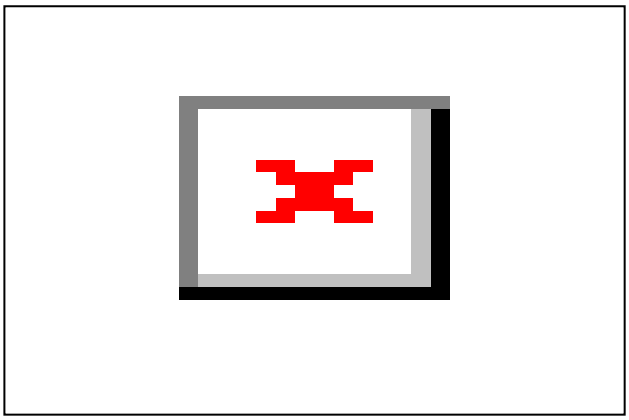
Test article-related clinical chemistry changes on Day 29 in the 40 mg/kg group included increases in ALT, AST, CK, and LDH (males and females); increases in alkaline phosphate, BUN, sodium and chloride (males), and decreases in total bilirubin, total protein and ALB (males and females); decreases in serum cholesterol and blood glucose (males); and decreases in serum globulin and increases in the mean ALB/globulin ratio in the 40 mg/kg females. Zotiraciclib (TG02) related clinical chemistry changes on Day 29 in the 20 mg/kg males included increases in AST, LDH, BUN, and triglycerides and decreases in bilirubin; and in the females there were decreases in globulin and increases in the ALB/globulin ratio and BUN value. Zotiraciclib (TG02)-related changes were present in the thymus, spleen, bone marrow, stomach, liver, testes, uterus, and/or small intestines of 10 mg/kg, 20 mg/kg, and 40 mg/kg scheduled termination mice, and consisted of atrophy and or necrosis of lymphoid tissues, reduced or increased hematopoiesis in the spleen, atrophy of bone marrow elements, testicular degeneration, small uterus, and atrophy and necrosis and/or regeneration (increased mitoses) of enterocytes in the small intestines. Zotiraciclib (TG02)-related lesions were reversed at recovery (Day 57) except in the GI tract, where minimal residual lesions were present in the small intestine and lesions were mildly increased in the forestomach. Additionally, testicular degeneration was still present in the 20 mg/kg mice at recovery.

In the confirmatory mutagenicity assay, no positive mutagenic response was observed. Zotiraciclib (TG02) citrate was evaluated for its genotoxic potential (clastogenicity/aneugenicity) as measured by its ability to increase the incidence of micronucleated polychromatic erythrocytes (MPCEs) in bone marrow of male and female mice. A significant increase in the incidence of MPCEs in bone marrow of male and female ICR mice received 100mg/kg Zotiraciclib (TG02), indicating the positivity in mouse micronucleus assay.

Clinical experiences

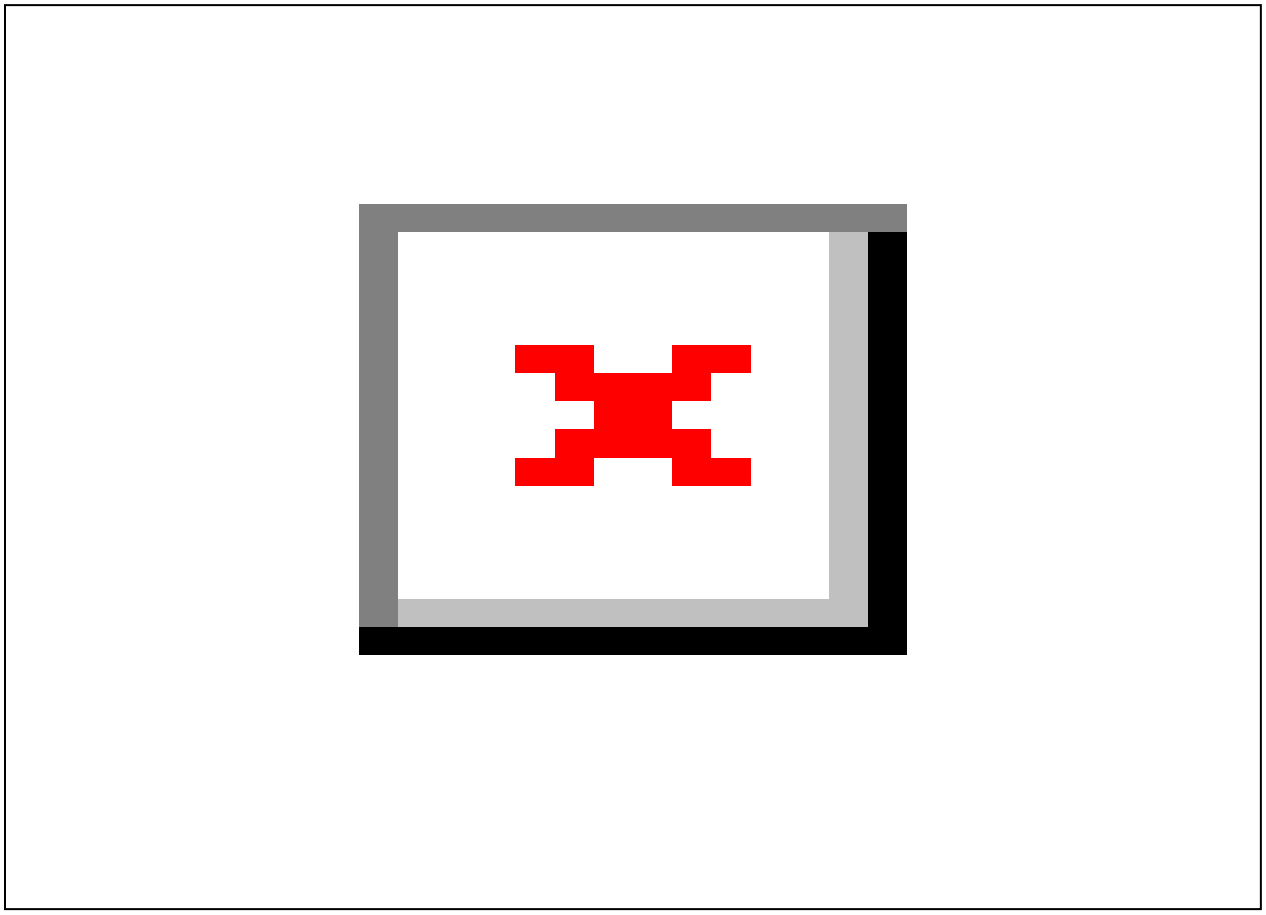
Two Phase 1 clinical studies with single agent Zotiraciclib (TG02) in hematological malignancy have been conducted (NCT01204164 and NCT01699152). The primary objective of these studies was to determine the MTD and DLT. Phase 1 study in combination with carfilzomib in multiple myeloma is ongoing. Additional study with Zotiraciclib (TG02) in combination with a hypomethylating agent is under development.

Figure 7: Zotiraciclib (TG02) 24-hour exposure in patients



The interim analysis of pharmacokinetic (PK) data from clinical studies has been evaluated for 55 acute leukemia patients, 18 multiple myeloma patients and 8 CLL patients. Mean exposures for Zotiraciclib (TG02) over twenty-four hours from Cycle 1 Day 1 (Figure 7) reveal dose-dependent increases in exposure (AUC0-t), C_{max} at approximately 2 hours and an estimated $t_{1/2}$ of 10 to 15 hours. Drug-related nausea and vomiting occur most frequently around C_{max} on dosing days.

Table 6: Summary of Zotiraciclib (TG02) Clinical Studies



Multiple consecutive daily doses resulted in Zotiraciclib (TG02) drug accumulation that was not predicted based on the nonclinical toxicokinetic studies. Drug accumulation was associated with higher incidence and frequency of exposure-related TEAEs, such as fatigue. Zotiraciclib (TG02) was administered three times per week (TIW) to multiple myeloma patients; TIW dosing reduced but did not alleviate drug accumulation. BIW dosing, twice per week, alleviated drug accumulation in all populations and reduced the incidence and severity of drug-related fatigue. Schedules of Zotiraciclib (TG02) clinical studies are summarized in Table 6 (Investigator's Brochure, Version 6). The preclinical pharmacodynamics data demonstrate that daily administration is not required to achieve anticipated therapeutic drug levels. Base on PK profiles, Zotiraciclib (TG02) will be administered no more than twice weekly (BIW).

In a Phase 1 study of Zotiraciclib (TG02) in multiple myeloma patients, a single agent Zotiraciclib (TG02) was administered to 18 multiple myeloma patients on three different schedules of a 28-day cycle: 6 patients administered Zotiraciclib (TG02) daily (3 patients at 50 mg and 70 mg each); 9 patients received Zotiraciclib (TG02) TIW on days 1, 3, 5, 8, 10, 12, 15, 17, 19 followed by a 9-day

rest (3 patients at 100 mg and 6 at 150 mg); 3 patients administered Zotiraciclib (TG02) BIW on days 1, 4, 8, 11, 15, 18 followed by a 10-day rest (all 3 patients at 200 mg). For the BIW schedule, all 3 patients successfully completed Cycle 1. No patient experienced a dose-limiting toxicity (DLT). At this time, it was decided to amend the protocol and pursue Zotiraciclib (TG02) in combination with carfilzomib. No further dose escalation was evaluated for Zotiraciclib (TG02) as a single agent and no MTD was identified.

An open label phase Ib study was developed with the primary objectives to determine the MTD of Zotiraciclib (TG02) in combination with carfilzomib (American Society for Hematology 2015 Abstract #3052). Zotiraciclib (TG02) was administered once daily on days 1, 4, 8, 11, 15, 18 of a 28-day schedule. The starting dose of Zotiraciclib (TG02) was 150 mg with dose escalation in 50 mg increments up to 300 mg. Carfilzomib was administered per the *Full Prescribing Information* or in accordance with institutional guidelines at the time of the study (i.e., dosing days 1, 2, 8, 9, 15 and 16 at 20 mg/m^2 for Cycle 1 and 27 mg/m^2 for Cycle 2 and subsequent cycles). Fourteen patients were enrolled for dose escalation and 10 patients were enrolled for the MTD cohort expansion. The MTD for Zotiraciclib (TG02) in combination with carfilzomib was established at 250 mg Zotiraciclib (TG02) administered twice a week for 3 weeks (Days 1, 4, 8, 11, 15, and 18) on a 28-days cycle. Two DLTs were observed, grade (Gr) 4 sepsis and neutropenia, both in 300 mg cohort. The most common drug-related adverse events were diarrhea (Gr 1-2: 4% and Gr3: 17%), nausea (Gr1-2: 79%), vomiting (Gr1-2: 50%), fatigue (Gr1-2: 38%, Gr3: 4%), anorexia (Gr: 21%), anemia (Gr1-2: 4%; Gr3:17%) and thrombocytopenia (Gr3: 8%, Gr4:13%).

Zotiraciclib (TG02) inhibited the hERG channel, suggesting a risk factor for torsade de pointes (QTc prolongation) in human. In the clinic, electrocardiographic QTc interval results for 73 patients enrolled in Parts 1 and 2 demonstrated that there were no clinically meaningful changes from baseline in resting 12-lead QTc intervals observed following administration of Zotiraciclib (TG02) on Cycle 1 Day 1 at 10 to 200 mg doses. Additionally, no clinically meaningful changes from baseline in resting 12-lead QTc intervals were observed following multiple doses of Zotiraciclib (TG02) at 10 to 200 mg doses through Cycle 1, regardless of schedule.

Patients with prolonged QTc intervals (males: >450 ms; females: >470 ms as calculated by Fridericia's correction formula) at screening/ baseline (based on the average QTc of triplicate ECGs), should be assessed for the following: normal electrolyte balance and concomitant medications known to prolong QTc. A patient can be reassessed for QTc interval after electrolytes are balanced and/or concomitant medications are stopped or replaced with an alternate medication. Patients, who continue to have prolonged QTc despite normal electrolyte balance and discontinuation of medications known to prolong QTc, will be excluded from the study. Use of concomitant medications that prolong QTc interval should be avoided if possible, during this study.

Zotiraciclib (TG02) may carry a risk of damage to chromosomes and should not be given to pregnant women or WOCBP not using an effective form of birth control. Men receiving Zotiraciclib (TG02) with female sexual partners of childbearing potential should use an effective form of birth control.

Zotiraciclib (TG02) may suppress bone marrow and the lymphoid system. Based on the

preliminary observation from the first 16 patients on the phase I portion of this study, moderate to severe neutropenia has been observed.

1.2.3 Rationale for using Zotiraciclib (TG02) in glioma treatment

High grade glioma is a common primary CNS tumor with a poor prognosis. Despite aggressive treatment, the disease eventually progresses, and the treatment options are often limited at the time of recurrence. There is a great unmet need to develop novel therapies in patients with recurrent high-grade glioma.

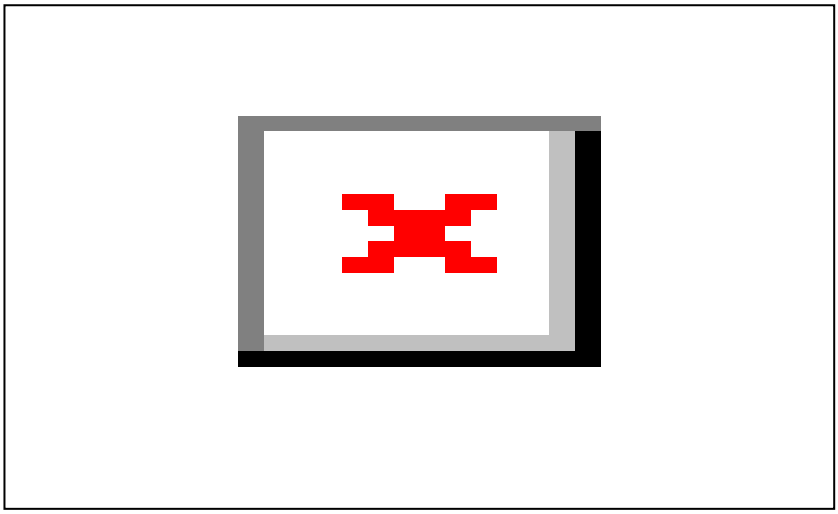
TMZ is an oral alkylating agent that has proven efficacy in AA and GBM. It has been proposed that the cytotoxic effect of TMZ is associated with induction of cell death or cell cycle arrest.[26] Mcl-1 was found to be essential for TMZ induced cell death.[27] Importantly, apoptosis was not found to be enhanced by ABT-737, an inhibitor of Bcl-2, but not Mcl-1. However, reducing expression of Mcl-1 increased TMZ-induced apoptosis, suggesting that targeting Mcl-1 enhances TMZ-induced cell death and therefore may improve response to TMZ treatment, even in patients with TMZ–refractory disease.

p53 is a transcription factor and has an important role in regulating cell cycle and apoptosis in response to a variety of cellular stress events. *TP53* mutation is a common genetic alteration in glioma. The incidence of *TP53* mutations is high in malignant gliomas and is reported to be higher in AA than GBM.[28] The mutant *TP53* has complex effects. There is not only the lack of normal p53 protein function, but there is also other mutation related gain-of-functions,[29] such as the ability to disrupt mechanisms that maintain cellular genome integrity and anti-apoptosis. This confers a selective survival advantage on cells within a competitive microenvironment. The ability to modulate Mcl-1 makes the rational to test Zotiraciclib (TG02) in an effort to enhance the apoptosis in glioma cells.

Furthermore, *MYC* gene amplification and MYC over-expression have been found in human glioblastoma.[30] MYC expression has an important role in cell growth and apoptosis. However, attempts to inhibit MYC haven't been successful. Therefore, there may be indirect mechanisms, through inhibition of CDK9 dependent transcription, to intervene in MYC oncogenic signaling pathway.

PTEN is a tumor suppressor that is frequently inactivated in brain tumors and inactivating *PTEN* mutations were found in 36% of glioblastoma based on a TCGA study.[31] Importantly, this was only found in primary but not secondary glioblastoma.[32] *PTEN* mutation is known to convey resistance to apoptosis-inducing treatments such as radiation and TMZ treatment, by upregulating anti-apoptotic proteins, including Mcl-1.[33] It was also shown that lack of PTEN can fuel the cell cycle by increasing the nuclear availability of cyclin D and thus promotes the transition from G1-phase to S phase of the cell cycle.[34]

Figure 8: The cell viability of patient-derived glioma cell lines is determined using Beckman Coulter Vi-CELL TM XR cell viability analyzer with ViCELL Quad Pak Kit. U251, U87, LN229, LN18 were obtained from ATCC and the sphere-forming GSC 923, GSC827 were developed from NOB laboratory. All cells were treated with different concentrations of Zotiraciclib (TG02) at 24-hour after the cell seeding and cell viability is detected following 72 hours culture.



The preliminary results of in vitro studies from our NOB laboratory and other laboratories have demonstrated that a subset of patient-derived glioblastoma cells is highly sensitive to Zotiraciclib (TG02). The IC_{50} of Zotiraciclib (TG02) are in the scale of nanomolar in the glioma cell lines that were studied (Figure **8**).

Figure 9: Western blot of several key molecular signals, reflecting the different genetic background of the cell lines that were used to study the effects of Zotiraciclib (TG02) treatments.

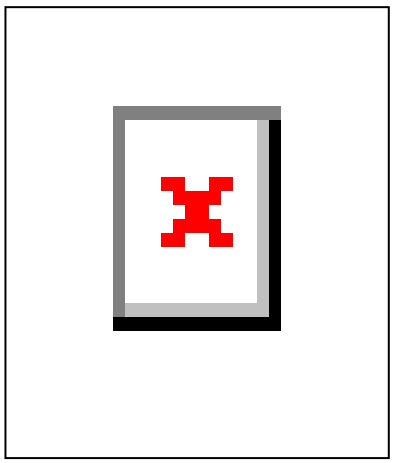
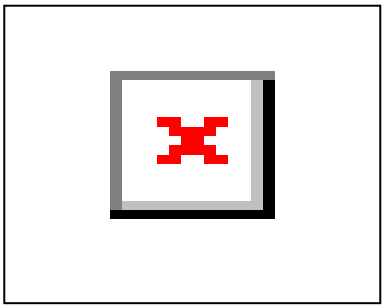


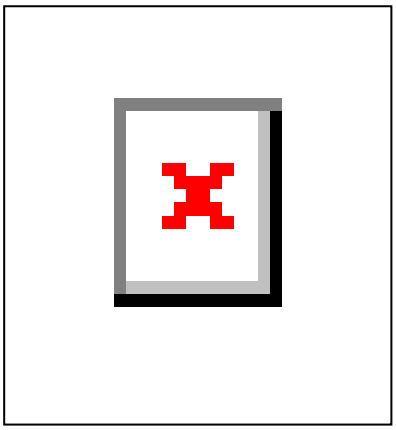
Figure 10: Western blot of cleaved Caspase 3, PARP and phospo-H2AX in GSC923 following treatment with Zotiraciclib (TG02) at 8, 16, 24, and 48 hours.



The cells that were tested have a wide range of genetic alterations as demonstrated in Figure 9. The high sensitivity to a variety of glioblastoma cell lines suggested that the board spectrum of effects of Zotiraciclib (TG02) in treating high grade glioma, which is known for inter-tumoral heterogeneity. This provides the opportunity to allow enrichment of molecular markers through carefully designed clinical trials. GSC923, a patient derived stem-like cell line developed in NOB laboratory was selected as a cell model to investigate the apoptotic effect of Zotiraciclib (TG02) in glioma. GSC923 has MGMT expression, which makes it insensitive to TMZ. It is also featured by *TP53* mutation and *PTEN* mutation. High c-Myc expression was also detected in this cell line. The genetic makeup makes GSC923 an appropriate *in vitro* model to study the biology of high grade astrocytoma. Caspase 3 activation was detected by both flow cytometry and Western blot in GSC923 following Zotiraciclib (TG02) treatment. Along with the presence of cleaved Poly (ADP-ribose) polymerase (PARP) and phosphorylated histone H2AX, the data suggested that Zotiraciclib (TG02) can induce apoptosis in glioma cells (Figure 10). As early as 8 hours after treatment with Zotiraciclib (TG02) at 50 nM, CDK9 expression was shown to be decreased and further decreased expression was detected at 48 hours following Zotiraciclib (TG02) treatment (Figure 11). The expression of anti-apoptotic proteins including survivin, XIAP, Mcl-1decreased along with the CDK9 inhibition following Zotiraciclib (TG02) treatment. The cell cycle analysis in GSC923 demonstrated the G2 arrest.

In summary, the potential to inhibit CDK9 activity leading to a subsequent apoptotic effect and modulation of cell cycle and other tyrosine kinases in cancer signaling pathways makes Zotiraciclib (TG02) a logical therapeutic agent to be tested in treatment of high grade astrocytoma. As described above, high-grade gliomas are known to harbor multiple genetic alterations, such as T*P53* mutation, *MYC* amplification and PTEN that can lead to resistance to apoptosis and treatment failure. Zotiraciclib (TG02) is orally administered and penetrates BBB with good concentration in the brain. The pharmacological inhibition of CDKs with optimized pharmacokinetic features of Zotiraciclib (TG02) may provide a new therapeutic option for high grade gliomas.

Figure 11: Western blot of surviving, XIAP Mcl-1 and c-MyC in GSC923 following treatment with Zotiraciclib (TG02) at 8, 16, 24, 48 hours.



1.2.4 Rationale for Zotiraciclib (TG02) starting dose

The starting dose of Zotiraciclib (TG02) was decided based on the results of an open label phase Ib study that was developed with the primary objectives to determine the MTD of Zotiraciclib (TG02) in combination with carfilzomib (American Society for Hematology 2015 Abstract #3052). In that study, Zotiraciclib (TG02) was administered once daily on days 1, 4, 8, 11, 15, 18 of a 28-day schedule. The starting dose of Zotiraciclib (TG02) was 150mg with dose escalation in 50 m increments up to 300mg. Carfilzomib was administered according to the *Full Prescribing Information* or in accordance with institutional guidelines at the time of the study (i.e., dosing days 1, 2, 8, 9, 15 and 16 at 20mg/m^2 for Cycle 1 and 27mg/m^2 for Cycle 2 and subsequent cycles). Fourteen patients were enrolled for dose escalation and 10 patients were enrolled for the MTD cohort expansion. The MTD for Zotiraciclib (TG02) in combination with carfilzomib was established at 250mg Zotiraciclib (TG02) administered twice a week for 3 weeks (Days 1, 4, 8, 11, 15, and 18) on a 28-days cycle. Carfilzomib is a selective proteasome inhibitor being evaluated for the treatment of relapsed and refractory multiple myeloma. In an open-label, single-arm phase 2 study (PX-171-003- A1 #NCT00511238), patients received single-agent carfilzomib 20 mg/m2 intravenously twice weekly for 3 of 4 weeks in cycle 1, then 27 mg/m2 for $\leqslant$ 12 cycles. Adverse events (AEs) were found to be manageable without cumulative toxicities.[35] Common AEs were fatigue (49%), anemia (46%), nausea (45%), and thrombocytopenia (39%). Thirty-three patients (12.4%) experienced peripheral neuropathy, primarily grades 1 or 2. Based on the *Full Prescribing Information* of temozolomide, more severe and frequent hematologic toxicities were described. In anticipation of more toxicities in patients receiving combination treatment with Zotiraciclib (TG02) and TMZ comparing to those receive Zotiraciclib (TG02) and carfilzomib, we decided to set the starting dose of Zotiraciclib (TG02) relatively lower and dosing less frequently followed by careful dose escalation, if the treatment is proved to be well tolerated. The starting dose of Zotiraciclib (TG02) is at 200 mg/day orally on days 1, 12, 15, and 26 on a 28-days cycle in both treatment arms, except for that the first dose of Zotiraciclib (TG02) will be given 3 days prior to Day1 in cycle 1. TMZ is

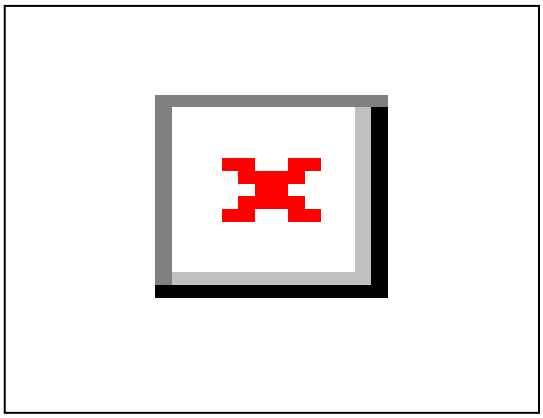
administered at 125mg/m^2 daily on days 1-7 and 15-21 orally in dd TMZ schedule and 50mg/m^2 daily in mn TMZ schedule. The dosing schedule was also designed with the intention to avoid potential overlapping toxicity from both drugs. The proposed dosing schedule will allow patients have less chance to have to take both medications on the same day. Based on our preclinical data that Zotiraciclib (TG02) reduces expression of anti-apoptotic protein and better anti-glioma effects with pretreatment of Zotiraciclib (TG02) and followed by combination treatment, one dose of Zotiraciclib (TG02) will be administered 3 days prior to first dose of TMZ in the first cycle. In the subsequent cycles, the doses that patients receive on day 26 would be the exactly 3 days prior to TMZ administration on day 1 of the following cycles.

1.2.5 Rationale for combination treatment with TMZ

TMZ is an alkylating agent that has been used in malignant gliomas of all grades. It produces *O6*-methylguanine in DNA, which mispairs with thymine during the next cycle of DNA replication. Subsequent futile cycles of DNA mismatch repair lead to a p53-associated apoptotic cell death.

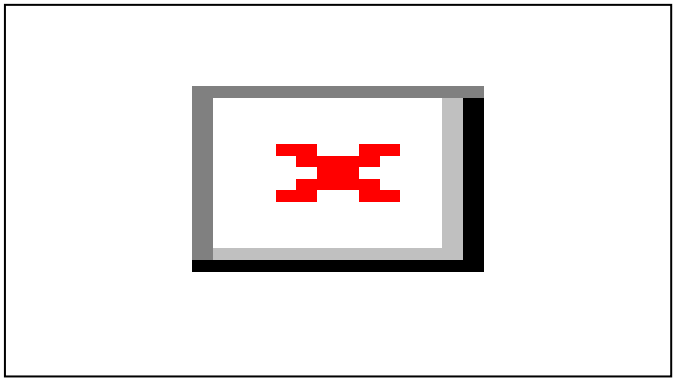
A seminal study from 2005 provided level 1 evidence that the addition of TMZ to radiotherapy for newly diagnosed glioblastoma resulted in a clinically meaningful and statistically significant survival benefit.[2] Since then, TMZ has become part of standard treatment for glioblastoma. Not only used in newly diagnosed glioblastoma, TMZ has been also frequently used in grade II and III gliomas, in both upfront and recurrent settings. Clinical studies in recurrent high grade gliomas also demonstrated objective response to TMZ.[4, 36] However, the treatment effects are always limited by the resistance mechanisms, including not only MGMT expression, but also p53 and p21 status when the mismatch repair mechanism is intact.[37] In gliomas with intact p53 function, TMZ can cause prolonged cell cycle arrest and apoptosis. However, in gliomas with *TP53* mutation, TMZ induced cell cycle arrest is not sustained.[38] Zotiraciclib (TG02) not only promotes apoptosis by decreasing expression of survival proteins, such as Mcl-1, but also by regulating the cell cycle by inhibiting CDKs. This broad spectrum of mechanisms of action makes Zotiraciclib (TG02) a strong candidate to overcome resistance to TMZ treatment and subsequently lead to a prolonged response to treatment.

Figure 12: Cell viability analysis of A. GDC923; and B. U251 by using cell counting following treatment with Zotiraciclib (TG02) and TMZ alone or in combination with different sequence: Zotiraciclib (TG02) treatment for 6 hours followed by TMZ; TMZ treatment for 6 hours followed by Zotiraciclib (TG02).



The preclinical study of cell proliferation assay from Neuro-Oncology Branch (NOB) laboratory using human-derived glioblastoma cell lines have demonstrated a significant additive effect of inhibiting tumor cell growth with combination treatment of Zotiraciclib (TG02) and TMZ. This additive effect was observed in both GSC and non-GSC cell lines (Figure **12**). In *in vitro* study, GSC923 was served as a model of high grade glioma with *MGMT* promotor unmethylation and U251 was served as a model of glioma with methylated *MGMT* promotor. Cell lines with different MGMT promotor methylation status respond to combination treatment differently, suggesting the role of MGMT methylation as a stratification factor in the randomization of the clinical trials that is designed to test clinical efficacy of combination treatment of Zotiraciclib (TG02) and TMZ in high grade gliomas. Our cell-based assay also suggests that the pretreatment with Zotiraciclib (TG02) followed by a combination treatment with TMZ cause better anti-glioma effects comparing to the opposite treatment sequence. This is consistent with the hypothesis that Zotiraciclib (TG02) enhances the apoptotic effects of TMZ by suppressing the expression of anti-apoptotic proteins, which is supported by the data demonstrated in Figure **13**. In Figure **13**, more decreased expression of survivin, XIAP and Mcl-1 is demonstrated after combination treatment with Zotiraciclib (TG02) and TMZ, comparing to the single treatment with either Zotiraciclib (TG02) or TMZ. These anti-apoptotic proteins seem to slightly induce by TMZ treatment, which potentially contributes to the treatment resistance.

Figure 13: Western blot Western blot of survivin, XIAP and Mcl-1 in GSC923 following treatment with Zotiraciclib (TG02), TMZ alone and in combination at 8, 16, 24, and 48 hours.



1.2.6 Rationale to test Zotiraciclib (TG02) in combination with TMZ with two different dosing schedules

Once patients with glioblastoma have recurrent disease after standard treatment defined as concurrent radiation plus daily TMZ followed by 6-12 cycles of adjuvant TMZ (150-200 mg/m2 days 1-5 of a 28-day cycle), the disease is typically less responsive to TMZ. This resistance has been attributed to an increase in the tumor intracellular concentration of the enzyme O6-methlyguanine DNA methyltransferase (MGMT). It has been demonstrated that if the MGMT can be depleted, sensitivity to alkylating agents such as TMZ can be restored. A study by Tolcher and his colleagues demonstrated that there was a more pronounced depletion of MGMT in peripheral blood mononuclear cells (PBMCs) after dose-intense dosing schedule of TMZ.[39] Building on this data, many studies explored the use of alternative schedules. The most notable evaluated the 7-day on – 7-day off and a metronomic schedule that used daily continuous dosing of 50 mg/m2 of TMZ.

Dose-dense schedule: In a prospective study, ninety adult patients with recurrent glioma were treated with TMZ at 150mg/m2/day on days 1-7 and 15-21.[40] PFS6 was reported to be 43.8% and one-year survival rate was 23%. In contrast, another study in a similar patient population reported a PFS6 of 10% and median overall survival was 21.6 weeks.[41] Both studies demonstrated good tolerance of the treatment.

Metronomic dosing: A study of recurrent glioma patients who received metronomic TMZ at 50mg/m2 yielded a PFS6 of 23.9% and a 1-year survival rate of 27.3% in recurrent glioblastoma and a PFS6 of 35.7% and a 1-year survival of 60.7% in recurrent anaplastic astrocytoma patients.[42] TMZ with both the dose-dense (dd) and metronomic (mn) schedules showed promising responses in recurrent high grade glioma patients. However, the superiority of either dosing schedule hasn't been established and comparison within the same clinical trial setting is therefore warranted.

1.2.7 Rationale for pharmacokinetic, pharmacogenetic and neutrophil function assay

Since the clinical trial 17C0009 was open to accrual, an unexpected phenomenon has been observed on multiple occasions- neutropenia with quick onset and quick recovery. Per current protocol, the hematologic toxicities have been mainly attributed to TMZ which is known for the bone marrow toxicities, including thrombocytopenia, leukopenia, neutropenia and lymphopenia. The complete blood count (CBC) of more than one patient happened to be tested based on clinical needs at several occasions. In those tests, absolute neutrophil count (ANC) in some patients was found to be decreased significantly. Interestingly, the low ANC can be found to be recovered within 24-48 hours in most cases. In one case, patient was found to have grade 4 neutropenia within 24 hours after one dose Zotiraciclib (TG02) but recovered to grade 1 neutropenia 24 hours later. The quick onset and quick recovery suggested that the bone marrow dysfunction is unlikely to be the etiology for the neutropenia that was observed in these cases. In addition, in most cases where this transient neutropenia occurred, there were no decreases in lymphocytes, platelets and hemoglobin. Although there were no serious consequences, such as infection in these cases, study drug had to be discontinued. Without knowing the pathophysiology of this type of neutropenia, the study drug can be discontinued prematurely. On the other hand, patients will be at risk if the follow up lab test**s** are not scheduled appropriately. It would be important to understand the effect of Zotiraciclib (TG02) on neutrophil biology.

To analyze the neutrophil function after Zotiraciclib (TG02) administration, information from pharmacokinetic study is crucial. To further understand why this neutropenia only happened in a subgroup of patients but not all patients receiving Zotiraciclib (TG02), we will add pharmacogenetic study by analyzing the genomic DNA and assess genotype of the most relevant drug metabolizing enzymes and transporters (DMET).

1.2.8 Rationale for the patient-reported outcome

Precedence for measuring “non-therapeutic” endpoints exists in oncology research. For example, Gemcitabine was approved by the FDA partially as a consequence of the decrease in pain reported in pancreatic patients who were treated, not on the basis of survival improvement, which was modest, at best. There have been efforts in neuro-oncology to evaluate secondary endpoints using validated instruments as an additional indicator of benefit.

The M.D. Anderson Symptom Inventory-Brain Tumor Module (MDASI-BT) allow the self-reporting of symptom severity and interference with daily activities. The MDASI-BT have demonstrated reliability and validity in the adult primary brain tumor patient population[43, 44]

This tool represents a modification of the widely used and validated M.D. Anderson Symptom Inventory, with particular attention to symptoms common in patients with brain tumors. The availability of validated instruments provides an opportunity to prospectively assess the impact of treatment, both positive and negative, on patients. The evaluation of symptom burden in this study will assist in finding the best possible treatment with the least toxicity.

1.2.9 Rationale for utilizing PRO-CTCAE

PRO-CTCAE is a patient-reported outcome measurement system developed by the NIH to capture the symptomatic adverse events in patients on cancer clinical trials. It was designed as a companion to the Common Terminology Criteria for Adverse Event (CTCAE), which is often used per medical professionals' evaluation. This instrument will allow us to select, but not be limited to the symptoms that are anticipated based on the previous experiences. It can be a flexible tool for descriptive reporting system, which patients can complete it at their own convenience. Since the timing for assessments are within 7 days, it certainly avoids the recall biases. It also can be used in conjunction with the CTCAE to provide a better understanding of the toxicities of the clinical trial treatments.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

Inclusion criteria are same in both Phase I and Phase II parts, except for the number of prior disease relapses in **2.1.1.5**.

2.1.1.1 Patients must have pathologic diagnosis of anaplastic astrocytoma (defined as WHO grade III, or glioblastoma/gliosarcoma, WHO grade IV, which are confirmed by NCI Laboratory of Pathology. If the pathology diagnosis is anaplastic glioma or anaplastic oligoastrocytoma, evidence of either intact 1p/19q chromosomes or molecular features suggesting astrocytic tumor must be present. (including, but not limited to ATRX, *TP53*).

2.1.1.2 Patients must have recurrent disease, histologically proven or imaging suggestive of recurrent disease as determined by PI. Prior implantation of Gliadel wafers is acceptable, if tumor recurrence is confirmed by histologic examination of the recurrent tumor.

2.1.1.3 Patients must have the ability to understand and the willingness to sign a written informed consent document.

2.1.1.4 Patients must be ≥18 years old.

2.1.1.5 No more than two prior disease relapses to be eligible for the phase I portion of the study and no more than one prior relapse to be eligible for phase II.

2.1.1.6 Patients must have undergone prior standard therapy for their primary disease. For patients with glioblastoma, this would include surgical resection or biopsy, if safe resection was not permitted due to the tumor location, radiation and adjuvant temozolomide. For patients with anaplastic astrocytoma, this would include surgical resection, radiation and adjuvant chemotherapy (PCV or temozolomide).

2.1.1.7 Tumor tissue must be available for review to confirm histological diagnosis.

2.1.1.8 Tumor block or unstained slides must be available for molecular profiling.

2.1.1.9 Karnofsky > 60%, (see **15.1**.)

2.1.1.10 Patients must have adequate bone marrow function (ANC > 1,500/mm3, platelet count of > 100,000/mm3), adequate liver function (ALT and AST< 3 times upper limit normal and alkaline phosphatase < 2 times upper limit normal, total bilirubin < 1.5mg/dl), and adequate renal function (BUN < 1.5 times institutional normal and serum creatinine < 1.5 mg/dl) prior to registration. These tests must be performed within 14 days prior to registration. Total bilirubin: patients with Gilbert's Syndrome are eligible for the study. (Total bilirubin level can be exempted from the eligibility criterion.)

2.1.1.11 Patients must have recovered from the toxic effects of prior therapy to < grade 2 toxicity per CTC version 4 (except deep vein thrombosis)

2.1.1.12 At the time of registration, subject must be removed from prior therapy as follows:

- ≥ 28 days from any investigational agent,
- ≥ 4 weeks (28 days) from prior cytotoxic therapy,
- ≥ 2 weeks (14 days) from vincristine,
- ≥ 6 weeks (42 days) from nitrosoureas,
- ≥ 3 weeks (21 days) from procarbazine administration,
- ≥ 1 week (7 days) for non-cytotoxic agents, e.g., interferon, tamoxifen, thalidomide, cis-retinoic acid, etc. (radiosensitizer does not count).

2.1.1.13 Patients having undergone recent resection of recurrent or progressive tumor will be eligible given all of the following conditions apply:

A. At least 2 weeks (14 days) have elapsed from the date of surgery and the patients have recovered from the effects of surgery.

B. Evaluable or measurable disease following resection of recurrent malignant glioma is not mandated for eligibility into the study.

C. To best assess the extent of residual disease post-operatively, an MRI should be done no later than 96 hours in the immediate post-operative period or at least within 4 weeks post-operatively, within 14 days prior to registration. If the 96-hour scan is more than 14 days before registration, the scan needs to be repeated. The patient must have been on a stable steroid dose for at least 5 days prior to the baseline MRI. Steroids may be initiated as clinically indicated once baseline imaging has been completed with a goal of titrating steroids as soon as clinically warranted.

2.1.1.14 Patients must have received prior radiation therapy and must have an interval of greater than or equal to 12 weeks (84 days) from the completion of radiation therapy to study entry except if there is unequivocal evidence for tumor recurrence (such as histological confirmation or advanced imaging data such as PET scan) in which case the principal investigator's discretion may determine appropriate timepoint at which study therapy may begin.

2.1.1.15 Women of childbearing potential must have a negative β-HCG pregnancy test documented within 14 days prior to registration. The effects of Zotiraciclib (TG02) on the developing human fetus are unknown. For this reason, women of childbearing potential must not be pregnant, must not be breast-feeding, and must practice adequate contraception (see **15.2** for details and definitions) for the duration of the study, and for

30 days after the last dose of study medication.

2.1.1.16 Male patients on treatment with Zotiraciclib (TG02) must agree to use an adequate method of contraception for the duration of the study, and for 30 days after the last dose of study medication as the effects of Zotiraciclib (TG02) on the developing human fetus are unknown (see **15.2** for definition of adequate methods of contraception.

2.1.1.17 Patients must agree to enroll on the NOB Natural History protocol to allow the assessment of molecular tumor markers.

2.1.2 Exclusion Criteria

2.1.2.1 Patients who are receiving any other investigational agents. However, prior enrollment on a study using investigational agents is acceptable as per section **2.1.1.12**.

2.1.2.2 Patients with prior bevacizumab use for tumor treatment. Patients who received bevacizumab for symptom management, including but not limited to cerebral edema, pseudo progression can be included in the study. (To date, there have been no effective regimens developed for recurrent malignant gliomas that are refractory to bevacizumab. Inclusion of this patient population may impact the ability to determine the efficacy of Zotiraciclib [TG02] with TMZ.)

2.1.2.3 Any serious medical condition, laboratory abnormality, or psychiatric illness that would prevent the subject from providing informed consent.

2.1.2.4 Any condition, including the presence of clinically significant laboratory abnormalities, which places the patient at unacceptable risk if he/she were to participate in the study or confounds the ability to interpret data from the study. These would include:

2.1.2.4.1 Active infection (including persistent fever) including known history of HIV or Hepatitis C infection, because these patients are at increased risk of lethal infections when treated with marrow-suppressive therapy.

2.1.2.4.2 Diseases or conditions that obscure toxicity or dangerously alter drug metabolism.

2.1.2.4.3 Serious concurrent medical illness (e.g. symptomatic congestive heart failure).

2.1.2.5 History of allergic reactions attributed to compounds of similar chemical or biologic composition to temozolomide and/or Zotiraciclib (TG02).

2.1.2.6 Patients with a history of any other cancer (except non-melanoma skin cancer; or melanoma in-situ following curative surgical resection; or carcinoma in-situ of the cervix or bladder), unless in complete remission and off all therapy for that disease for a minimum of 3 years, are ineligible.

2.1.2.7 Zotiraciclib (TG02) is primarily metabolized by CYP1A2 and CYP3A4. Patients receiving any medications or substances that are strong inhibitors or inducers of *CYP1A2 and/or CYP3A4* are ineligible. Lists including medications and substances known or with the potential to interact with the CYP1A2 and CYP3A4 isoenzymes are provided in **15.3.**

2.1.2.8 Patients, who continue to have prolonged QTc (males: >450ms; females: >470ms as calculated by Fridericia's correction formula) despite normal electrolyte balance and discontinuation of medications known to prolong QTc, will be excluded from the study.

2.1.3 Recruitment Strategies

This protocol may be abstracted into a plain language announcement posted on NIH websites and on NIH social media platforms.

2.2 SCREENING EVALUATION

2.2.1 Screening activities performed prior to obtaining informed consent

Minimal risk activities that may be performed before the subject has signed a consent include the following:

- Email, written, in person or telephone communications with prospective subjects
- Review of existing medical records to include H&P, laboratory studies, etc.
- Review of existing MRI, x-ray, or CT images
- Review of existing photographs or videos
- Review of existing pathology specimens/reports from a specimen obtained for diagnostic purposes

A waiver of consent for these activities has been requested in section **12.6.1**.

2.2.2 Screening activities performed after a consent for screening has been signed

The following activities will be performed only after the subject has signed the study consent OR the consent for study 01-C-0129 (provided the procedure is permitted on that study) on which screening activities may also be performed. Assessments performed at outside facilities or on another NIH protocol within the timeframes below may also be used to determine eligibility once a patient has signed the consent.

To be completed within 14 days of study registration, unless otherwise indicated

2.2.3 Unstained paraffin tissue from surgical samples will be obtained. A representative paraffin tissue block (at least 5 mm x 5 mm) or unstained slides will be obtained from previous surgery to confirm the histological diagnosis and molecular markers before entry into the clinical trial.

2.2.4 All patients with recurrent anaplastic astrocytoma or glioblastoma/gliosarcoma will need to have confirmation of pathology diagnosis on initial disease or recurrent disease, by a Neuropathologist at the NIH. The recurrent status of the disease must be confirmed by either by pathology diagnosis on tissue from recurrent disease or by imaging review by the PI. Confirmation of pathology diagnosis may occur any time prior to study entry.

2.2.5 A Gd-DPTA enhanced MRI, with DWI, ADC, SWI, FLAIR, perfusion, dynamic contrast enhanced, and dynamic susceptibility contrast sequences is needed within 14 days of entry into the study in all patients without surgical resection to establish the diagnosis of recurrent disease. The treating physician will review the Gd-DPTA MRI scan done prior to study entry documenting progression, if no tumor tissue available for pathology diagnosis.

2.2.6 Complete history and physical exam completed by principal or associate investigator, including thorough documentation of signs and symptoms caused by the tumor, determination of KPS, neurological function and mental status.

2.2.7 Screening laboratory tests will include CBC, differential, platelets, PT, PTT, INR, total protein, albumin, calcium, phosphorus, magnesium, glucose, BUN, creatinine, sodium, potassium, total bilirubin, alkaline phosphatase, LDH, SGPT (ALT), SGOT (AST), 12-lead EKG and a pregnancy test for women of child bearing potential.

2.3 PARTICIPANT REGISTRATION AND STUDY STATUS UPDATE PROCEDURES

Registration and status updates (e.g. when a participant is taken off protocol therapy and when a participant is taken off-study) will take place per CCR SOP ADCR-2, CCR Participant Registration & Status Updates found here.

Patients must initiate study treatment within 7 calendar days after registration.

2.3.1 Treatment Assignment and/or Randomization/ Stratification Procedures*

Cohorts

Number	Name	Description
1	Cohort 1	Patients with recurrent anaplastic astrocytoma or glioblastoma/gliosarcoma assigned to Phase 1

2	Cohort 2	Patients with recurrent anaplastic astrocytoma or glioblastoma/gliosarcoma assigned to Phase 2

Arms

Number	Name	Description
1	Phase I Arm I	dose dense TMZ+ Zotiraciclib (TG02)
2	Phase I Arm 2	metronomic TMZ+ Zotiraciclib (TG02)
3	Phase II Arm 1	TMZ + Zotiraciclib (TG02)
4	Phase II Arm 2	TMZ (Control Arm)

Stratifications

Name	Distinct Options
KPS	Yes No
MGMT promoter methylation status of the tumor	Yes No
Presence of Measurable Disease	Yes No

In Phase I, study patients will be assigned to one of two arms to receive Zotiraciclib (TG02) plus dd TMZ or Zotiraciclib (TG02) plus mn TMZ in 1:1 ratio*. Once a phase II dose/schedule is determined, patients will be randomized to the following arms in 1:1 ratio - Zotiraciclib (TG02) plus TMZ (dose and schedule selected from phase 1 component) or Control Arm – TMZ (dosing schedule will match combination arm). Patients' KPS, MGMT promoter methylation status of the tumors and the presence of measurable disease at study entry will be used as stratification factors.

*In Phase I, Post-interim analysis patients will be registered to cohort 1 and assigned to ARM 1, at a TG02 dose level of 200mg. Patients will be enrolled consecutively to cohort 1. Details are described in Section **3.2.1.3**.

2.4 BASELINE EVALUATION

Baseline tests do not need to be repeated if the same tests were done within 14 days of treatment initiation.

2.4.1 A complete history and physical (including baseline blood pressure measurement) and neurological examination (include documentation of the patients' height, weight and Karnofsky Performance Status), as well as documentation of measurable and/or evaluable disease shall be performed on all patients.

2.4.2 A baseline Gd-DPTA enhanced MRI, with DWI, ADC, SWI, FLAIR, perfusion, dynamic contrast enhanced and dynamic susceptibility contrast sequences should be performed. The baseline on-study MRI should be performed on a steroid dosage that has been stable or decreasing for at least 5 days. If the steroid dose was increased between the date of imaging and the initiation of therapy (or at that time), a new baseline MRI is required. MRI study needs to be done within 14 days of the initiation of the study treatment.

2.4.3 Pre-treatment laboratory tests will include CBC, differential, platelets, PT, PTT, INR, total protein, albumin, calcium, phosphorus, magnesium, glucose, BUN, creatinine, sodium, potassium, total bilirubin, alkaline phosphatase, LDH, SGPT (ALT), SGOT (AST), 12-lead EKG and a pregnancy test for women of child bearing potential. Blood tests must be performed within 14 days (+ 3 working days) of registration, with day 0 = registration date. Pregnancy test must be obtained 14 days (+ 3 working days) before treatment starts.

2.4.4 Patients will complete a baseline MD Anderson Symptom Inventory-Brain Tumor Module (MDASI-BT) (**Error! Reference source not found.**). The MDASI-BT will only be completed by the patient, unless changes in vision or weakness make this difficult. If this occurs, then the caregiver or research assistant may read the questions to the patient or assist with marking the severity number or score as described by the patient. A patient caregiver may complete the questionnaires as a patient-preference proxy if the patient's deficits preclude self-report. If the MDASI-BT is completed by the patient for Natural History study, 16C0151 within (+/-) 14 days from the initiation of the treatment, then it can be used as the baseline.

2.4.5 Rapid clinical deterioration from the time of study entry to initiation of treatment will mandate a full re-evaluation including physical examination, KPS, MRI, and laboratory studies including CBC and blood chemistries and MDASI as outlined in Sections 2.5.1 - 2.4.4.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN:

3.1.1 Phase I:

The phase I component is designed to find the MTD of Zotiraciclib (TG02) in combination with 2 schedules of TMZ – dose dense and metronomic. Patients will be enrolled and equally assigned into the two dosing schedule arms for MTD finding. In each arm, the MTD will be determined independently using the Bayesian Optimal Interval (BOIN) design, fully described in Section **8** and recently published [45]. Once the MTDs of the two regimens (dd TMZ + Zotiraciclib (TG02) and mn TMZ + Zotiraciclib [TG02]) have been established, cohort expansion will be carried out at the each of the MTDs. Patients will be equally randomized into to the two cohort expansions. A post interim analysis cohort will be carried out in cohort 1 arm 1 to confirm MTD. The MTD that has higher progression-free survival rate at 4 months (PFS4) will be selected and used in phase II.

3.1.2 Phase II:

The two-arm randomized phase II will determine if the addition of Zotiraciclib (TG02) to the dd or mn TMZ schedule (determined by the expanded phase I) improves outcome in patients with recurrent malignant glioma using PFS as the primary outcomes measure. Patients will be equally randomized into the combination Arm (Zotiraciclib [TG02] plus TMZ selected from phase I) and control arm (TMZ). The randomization will stratify by the presence of measurable disease at the study entry, KPS and MGMT promoter methylation status. Additional outcomes such as PFS6, overall survival from study entry and symptom burden (using the MDASI-BT instrument in Appendix **Error! Reference source not found.**) will also be collected.

The study schema is illustrated in Study Flow Chart in Appendix **15.4.**

3.1.3 Definition of dose limiting toxicities (DLT)

All patients in the MTD finding phase of the phase I part will be evaluable for assessment of toxicity to define DLTs. The DLT-defining period is the first 4 weeks' treatment in the Phase I study.

Toxicities will be graded per the Common Terminology Criteria for Adverse events (CTCAE) Version 4.0. If multiple toxicities are seen, the presence of DLT should be based on the most severe toxicity experienced. DLT is defined as any of the following events occurring in the first cycle of treatment (4 weeks) and attributable to the study drugs:

- Any of the following hematological toxicities:
    - Grade 4 neutropenia lasting 5 days or more.
    - Febrile neutropenia defined as grade 3-4 neutropenia with fever ≥38.5ºC and/or infection requiring antibiotic or antifungal treatment.
    - Grade 4 thrombocytopenia lasting more than 2 days.
    - Grade 3 thrombocytopenia with bleeding of any duration

  - Grade 3 or 4 anemia lasting more than 2 days.
- Any non-hematologic grade 3 or 4 toxicity, except the following
  - nausea or vomiting that responds to symptomatic therapy and lasts ≤7 days.
  - Diarrhea that responds to symptomatic therapy within 2 days.
  - fatigue that responds to symptomatic therapy and lasts ≤7 days.
  - weight gain (in patients on steroids)
  - venous thromboembolic disease (rationale for not including this event as a DLT described below)
- If there is a dose reduction of either or both study medications or if a dose is held or discontinued in cycle 1, this constitutes a DLT, per PI discretion.
- Grade 4 lymphopenia is not considered as a DLT for this study. All patients for this study have treated with alkylating agents and radiation treatment. Lymphopenia can be highly associated with the previous treatment or have a low threshold to have severe lymphopenia because of the previous treatments. In case of isolated laboratory abnormalities that may not reach clinical significance, a determination will be made by PI or in consultation with the IRB on a case-by-case basis to determine if DLT needs to be declared.
- Failure to recover from treatment related toxicities to be eligible for re-treatment within 4 weeks of the last dose of the drugs.

3.1.4 Dose escalation

We will employ the Bayesian optimal interval (BOIN) design [46] to guide dose escalation and find the MTD. The details of the design are provided in Section **8.** There are two treatment arms in the Phase I study: the combination treatment with Zotiraciclib (TG02) and dd TMZ; and the combination treatment of Zotiraciclib (TG02) with mn TMZ. Dose escalation will be conducted independently in these two arms. Several dose levels of Zotiraciclib (TG02) are planned as outlined in the Dose Escalation Table below.

Table 7: Dose Escalation Table

	Arm 1: Dose-dense temozolomide	
Level	**TMZ (mg/m^2 x 7 days on/7 days off)****	**TG 02 (mg/day on 1, 12,15,26 per 28 day cycle)***
-1	125	150
0 (starting dose)	125	200
I	125	250
II	125	300***

	Arm 2: Metronomic temozolomide	
Level	**TMZ (mg/m^2 daily)****	**TG 02 (mg/day on 1, 12,15,26 per 28 day cycle)***
-1	50	150
0 (starting dose)	50	200
I	50	250
II	50	300***

* The first dose of Zotiraciclib (TG02) will be given 3 days prior to Day1 of cycle 1. The subsequent doses for cycle 1 will be given on days 1, 12, 15, and 26. Zotiraciclib (TG02) will be given on days 1, 12, 15, and 26 every 28-days cycle in all the cycles starting cycle 2.

**BSA (body surface area) for Temozolomide dosing calculation will be capped at 2.2m^2 to avoid excessive risk of myelosuppression.

*** One patient in MN arm developed severe toxicities which lead to ICU admission after one dose of Zotiraciclib (TG02) at 300mg. The etiology was not completely clear. However, due to the patient's safety concern, does level II of Zotiraciclib (TG02) was determined to be eliminated in both study arms by the investigator and pharmaceutical manufacturer.

3.2 DRUG ADMINISTRATION

3.2.1 Phase I treatment

3.2.1.1 Drug dosage

There are two treatment arms in this component: the combination treatment of Zotiraciclib (TG02) and dd TMZ; and the combination treatment of Zotiraciclib (TG02) with mn TMZ. In both treatment arms, several dose levels are planned as outlined in section **3.1.4.** The starting dose of Zotiraciclib (TG02) is 200 mg/day orally on days 1, 12, 15, and 26 on a 28-days cycle in both treatment arms, except for that the first dose of Zotiraciclib (TG02) will be given 3 days prior to Day1 in cycle 1. The dosing schedule for both treatment arms are illustrated in the Appendix **15.6**. TMZ is administered at 125mg/m^2 daily on days 1-7 and 15-21 orally in dd TMZ schedule and 50mg/m^2 daily in mn TMZ schedule. The dosing schedule was designed with the intention to avoid potential overlapping toxicity from both drugs. The proposed dosing schedule will allow patients have less chance to have to take both medications on the same day. Based on our preclinical data that Zotiraciclib (TG02) reduces expression of anti-apoptotic protein and better anti-glioma effects with pretreatment of Zotiraciclib (TG02) and followed by combination treatment, one dose of Zotiraciclib (TG02) will be administered 3 days prior to first dose of TMZ in the first cycle. In the subsequent cycles, the doses that patients receive on day 26 would be the exactly 3 days prior to TMZ administration on day 1 of the following cycles. We will employ the Bayesian optimal interval (BOIN) design [46] to guide dose escalation and find the MTD. The details of the design are provided in Section **8.**

3.2.1.2 Cohort expansion:

After identifying the MTD for each of the TMZ dosing schedules in combination with Zotiraciclib (TG02) (i.e., 125mg/m2 TMZ on days 1-7 and 15-21 per cycle, and 50 mg/m2 TMZ daily), cohort expansion will be conducted independently at each of the MTDs until the total number of patients treated at each of the MTDs reaches 18 patients (including the patients who have been treated in the dose-finding part). The dosing schedule with a higher PFS4 will be selected and used in phase II.

3.2.1.3 Post interim analysis cohort:

Patients will be enrolled to Arm 1 only to receive Zotiraciclib (at Dose level 0 and in combination of dd TMZ). TG02 dose is 200 mg/day orally on days 1, 12, 15, and 26 on a 28-days cycle, except for that the first dose of Zotiraciclib (TG02) will be given 3 days prior to Day1 in cycle 1. TMZ is administered at 125mg/m^2 daily on days 1-7 and 15-21 orally on a 28-days cycle.

3.2.2 Phase II treatment:

The combination treatment selected from phase I (with better PFS4) will be tested against the TMZ alone with the same dosing schedule as it is in the combination arm. Patients will be randomized on a 1:1 basis between two competing treatment arms: TMZ + Zotiraciclib (TG02) versus TMZ alone using a Bayesian phase II trial design. The details of the design are provided in Section **8.**

The dosage for the combination arm is derived from the MTD of the combination determined in the phase I component of the study. The treatment schedule will be identical to that described above in the phase I component, with each cycle comprising 28 days.

Patients will continue treatment until tumor progression or unacceptable toxicity occurs or completion of 12 cycles of treatment. At progression, patients randomized to the control arm (TMZ alone) will be offered the opportunity to continue TMZ and the additional treatment with Zotiraciclib (TG02). After administration of 12 cycles of treatment, patients may stay on the study treatment, if there is evidence of clinical benefit based on treating physician's discretion.

If patient vomits Zotiraciclib (TG02), do not re-administer.

3.3 DOSE MODIFICATION/DELAYS:

If an adverse event occurs wherein both TMZ and Zotiraciclib (TG02) would require dose de-escalation, the Principal Investigator shall have the discretion of first dose reducing one of the 2 study drugs with the consideration of previously known drug toxicities/side effects in an effort to further define attribution.

Table 8: Start of Each Cycle for both TMZ and Zotiraciclib (TG02)		
Temozolomide and Zotiraciclib **(TG02)**		
Hematologic*	ANC ≥ 1.5 x 10^9/L and PLT ≥ 100 x 10^9/L	No dose modification
	ANC< 1.5 x 10^9/L and/or Platelet count < 100 x 10^9/L	Delay up to 4 weeks until ANC ⩾ 1.5 x 10^9/L and PLT ⩾ 100 x 10^9/L. If unresolved after 4 weeks, then stop. If resolved, dose delay/reductions based on Table 9.
Non-Hematologic	Grade 1 or less	No dose modification
	Grade 2	Zotiraciclib (TG02): Delay up to 4 weeks until ≤ Grade 1. If unresolved after 4 weeks, then dose reduce by 1 dose level. Temozolomide: dose delay/reductions based on Table **9**, if adverse event attributed to Temozolomide.
	Grade 3	Zotiraciclib (TG02): Delay up to 4 weeks until ⩽ Grade 1. If unresolved after 4 weeks, then stop. If resolved to </= Grade 1, then dose reduce by 1 dose level. Temozolomide: dose delay/reductions based on Table **9**, if adverse event attributed to Temozolomide.
	Grade 4	Discontinue treatment

*With the exception of lymphopenia, grade 1-4. Patient may start a cycle with G2 WBC, as long as the ANC ≥ 1.5 x 10^9/L.

3.3.1 Temozolomide (TMZ) Dose Modifications

Dose modification(s) are based on adverse event occurrence and may be implemented during any treatment cycle (Table **11**) or prior to initiation of each new cycle per Table **8**. If multiple AEs are seen, the dose administered should be based on the dose reduction required for the most severe grade of any single AE. If TMZ is delayed or discontinued in combination therapy, the Zotiraciclib (TG02) component of treatment may continue, unless there is clear reason to discontinue Zotiraciclib (TG02) simultaneously.

Table 9: ***TMZ Dose Levels for Modification Table***		
Dose Level	**DD TMZ Dose, mg/m^2/day**	**MN TMZ Dose, mg/m^2/day**
-2	80	32
-1	100	40
0	125	50

3.3.1.1 Delay

For treatment on day 1 of each cycle (within the prior 72 hours), ANC ≥ 1.5 x 10^9/L, platelet count ≥ 100 x 10^9/L and all grade 2 -3 non-hematologic AEs (except nausea and vomiting) must have resolved (to grade ≤ 1). For grade 4, discontinue treatment. For the purposes of this study, any clinically insignificant Grade 2 or 3 electrolyte abnormalities with attribution of unlikely related to or unrelated to study medications, will not require holding or dose-reducing study medications.

If AEs persist, treatment may be delayed by 1 week for up to 4 consecutive weeks. If, after 4 weeks of delay, all AEs have still not resolved to grade 1 or less: then any further treatment with temozolomide should be stopped, as summarized in Table **8** and Table **11**.

3.3.1.2 Dose reductions (Summarized in Table **11**)

If any non-hematologic AE observed was grade 3 (except nausea and vomiting) and/or if platelets < 50 x 10^9/L and/or ANC < 1 x 10^9/L, then the dose should be reduced by one dose level. For patients who would require dose reductions to a dose level < -2 as defined in table 10, temozolomide will be stopped. Also, if any of the same non-hematologic grade 3 AE recurs (except, nausea and vomiting,) after reduction for that AE, then temozolomide will be stopped.

If any treatment-related non-hematologic AE observed was grade 4 (except nausea and vomiting) then adjuvant temozolomide treatment should be stopped.

Any dose reductions of temozolomide will be determined according to (1) non-hematologic AE, as well as (2) the lowest/worst ANC and platelet counts observed. No dose escalation should be attempted. If the dose reduction occurs in the middle of the cycle, the reduced dose should be applied as soon as the treatment resumes (i.e. next dose).

3.3.2 Zotiraciclib (TG02) Dose Modifications

If multiple AEs are seen, the dose administered should be based on the dose reduction required for the most severe grade of any single AE. Prior studies of Zotiraciclib (TG02) indicate that non-hematologic toxicities have been the dose-limiting toxicities. Therefore, in the combination regimens, attribution of treatment-related toxicities will be made based on best available information. As Zotiraciclib (TG02) has not been reported with high rates of prolonged myelotoxicity, these toxicities will likely be attributed to temozolomide and dose modifications

made as outlined in Section **3.3.1** above. Conversely, non-hematologic adverse events such as nausea and vomiting, intractable diarrhea, fatigue and prolongation of the QTc interval on ECG will be attributed to Zotiraciclib (TG02). Liver toxicities are a known toxicity of TMZ, which has been tested extensively in patients with high grade glioma. Zotiraciclib (TG02) has also been reported to cause elevated liver enzymes. To better understand the attribution of this toxicity, in the event of elevated ALT/ AST of grade 2 or above when a patient has received the combined treatment, dose modification will be made on one drug at a time. Should the need for dose modification arise again due to the same toxicity, dose of the second drug will be reduced. If a dose reduction occurs in the middle of the cycle, the reduced dose should be applied as soon as the treatment resumes.

Details for dose modification, delay at beginning of each cycle and during the cycle are summarized in Table **8** and Table **11** respectively.

The dose of Zotiraciclib (TG02) will be initiated at the phase I dose for patients enrolled on the dose-finding component or at the MTD for both the cohort expansion of the phase I and the phase II component of the trial. The same dose will be continued unless > or = grade 3 drug related toxicity occurs; treatment will be held until toxicity resolves to grade 1 or less. At that time, the dose will be reduced to one level below the dose that caused the grade 3 toxicity. Only two dose reductions will be allowed for Zotiraciclib (TG02). Patients enrolled prior to determination of the MTD will have stepwise reductions as necessary based on the Table **10** below. Please note, if the MTD is 150 mg/day, then no dose reduction will be allowed. Additionally, if the patient is treated at dose Level 0, only one dose reduction (to 150 mg/day) will be allowed.

Table 10: Zotiraciclib (*TG 02) Dose Modification Table*

Dose Level	**TG 02 (mg/day on days 1, 12, 15, 26 per 28 day cycle)***
-1	150
0	200
1	250
2	300

Summary of Dose Modification or Discontinuation

Table 11: *Dose Modifications and Delay**

	Zotiraciclib (TG02)		TMZ	
Grade	Hematologic**	Non-hematologic	Hematologic**	Non-hematologic
0-1	No dose modification	No dose modification	No dose modification	No dose modification

Table 11: *Dose Modifications and Delay**				
	Zotiraciclib (TG02)		TMZ	
2	Hold until resolved to ⩽ G1; if persists beyond 4 weeks, dose reduce one level.	Hold until resolved to ≤ grade 1; if > 4 weeks then dose reduce 1 level.	Hold until resolved to ≤ grade 1; if > 4 weeks then dose reduce 1 level.	No dose modifications for non-hematologic AEs (G 0-2), unless the adverse event attributed to Temozolomide.
3	If not recovered to ⩽ G2 within 3 days, STOP. If recovered to ⩽ G2 within 3 days, re-start @ one dose level lower once recovered to G1 or less.***	Delay up to 4 weeks until ≤ Grade 1. If unresolved after 4 weeks, then stop. If resolved to ≤ grade 1; then dose reduce by 1 level.	Hold until resolved to ≤ grade 1; if > 4 weeks, then stop.	Hold until resolved to ⩽ grade 2; if greater than 4 weeks then stop. If less than 4 weeks, reduce by one dose level. If the same non-hematologic grade 3 AE recurs after reduction for that AE, then stop.
4	If not recovered to ⩽ G2 within 3 days, STOP. If recovered to ⩽ G2 within 3 days, re-start @ one dose level lower once recovered to G1 or less	Stop treatment.	Stop treatment.	Stop treatment.

* If the patient develops a lower extremity deep venous thrombosis and requires anticoagulation or intervention (grade 2 or 3 toxicity), treatment may be restarted after a 2-week rest period if the patient is stably anticoagulated and is judged medically stable to begin chemotherapy. (Please note: DVT is graded in the CTC version 4.0 as grade 1= superficial thrombosis, grade 2= DVT, medical intervention indicated, grade 3= uncomplicated pulmonary embolism, medical intervention indicated, grade 4=life-threatening pulmonary embolism, cerebrovascular event, arterial insufficiency, urgent intervention indicated).

** With the exception of lymphopenia, grade 1-4.

***If G3 neutropenia recovers within 72 hours, the Principal Investigator may consider no dose reduction of Zotiraciclib (TG02).

If leukopenia is grade 2 or above while ANC is grade 1 or less, the principal investigator may not consider it for dose modification and delay.

3.3.3 Pre-medications

3.3.3.1 Zotiraciclib (TG02)

Patients must receive vigorous prophylactic treatment for nausea and vomiting. Prophylactic use of moderately anti-emetogenic treatments (e.g., 5-HT3 antagonist) has been more effective at controlling nausea and vomiting than lightly anti-emetogenic treatments or treatment after onset.

3.3.3.1.1 Prophylaxis for Nausea/Vomiting

Zotiraciclib (TG02) is potentially highly emetogenic. Vigorous anti-emetic prophylaxis is required prior to administration of Zotiraciclib (TG02) and should continue at least 24 hours after Zotiraciclib (TG02) administration or until nausea/vomiting returns to Grade 0 or 1. Anti-emetic prophylaxis is also highly recommended to be started on the day prior to Zotiraciclib (TG02) administration.

Some recommended prophylaxis regimens include but is not limited to the following:

- 5-HT3 antagonist (e.g., ondansetron, granisetron, palonosetron, etc.)
- 2.5 mg olanzapine or
- other moderate anti-emetic regimens tailored to the individual patient at the Investigator's discretion

3.3.3.1.2 Prophylaxis for Diarrhea

Due to frequent reports of diarrhea with Zotiraciclib (TG02) administration, vigorous anti-diarrheal prophylaxis (e.g., loperamide or equivalent) is required prior to administration of Zotiraciclib (TG02) and should continue at least 24 hours after Zotiraciclib (TG02) administration or until diarrhea returns to Grade 0 or 1.

The recommended prophylaxis includes but is not limited to the following:

a. Loperamide the day prior to and the day of Zotiraciclib (TG02). Suggest that if patient had diarrhea on day of Zotiraciclib (TG02) that Loperamide 2-4mg is administered following first loose stool on day after Zotiraciclib (TG02) and after each stool if an hour as elapsed since last dose, as needed thereafter.

b. Once diarrhea has occurred, Loperamide 4mg after first loose stool, and Loperamide 4mg after the second and third stool respectively, with patient to call the study team practitioner after third loose stool.

c. Consider Lomotil and Tincture of Opium, if diarrhea continues. A nutrition consult may also be beneficial for potential diet modifications.

3.4 QUESTIONNAIRES

3.4.1 MD Anderson Symptom Inventory-Brain Tumor (MDASI-BT):

The MDASI-BT will be administered at baseline and at the time of imaging analysis as outlined in the protocol. Of note, every attempt should be made to administer the MDASI-BT prior to informing the patients about the results of the imaging study. The MDASI-BT will be completed only by the patient, unless changes in vision or weakness make this difficult. If this occurs, then the caregiver or research assistant may read the questions to the patient or assist with marking the severity number or score as described by the patient. A caregiver may complete the questionnaires as a patient proxy if the patient's deficits preclude self-report; however, this must be done at every assessment from baseline to end of treatment. In addition, information regarding demographics and treatment history will be collected as part of the larger study and used in this analysis.

The MDASI-BT consist of symptoms rated on an 11-point scale (0 to 10) to indicate the presence and severity of the symptom, with 0 being "not present" and 10 being "as bad as you can imagine." Each symptom is rated at its worst in the last 24 hours. Symptoms included on the instrument include those commonly associated with cancer therapies, and those associated with the underlying disease. The questionnaire also includes ratings of how much symptoms interfered with different aspects of a patient's life in the last 24 hours. These interference items include: general activity, mood, work (includes both work outside the home and housework), relations with other people, walking, and enjoyment of life. The interference items are also measured on 0 - 10 scales. The average time to complete this instrument is 5 minutes. The MDASI-BT has been translated into multiple languages, but the English language version will be used for this study[44].

3.4.2 PRO-CTCAE*:

Patients will complete a baseline PRO-CTCAE any time after enrollment but before starting treatment, and again on days 2, 8, 14, 21, and 28 in each treatment cycle, if the patient is able. There are 13 questions in total. The questions are focused on 12 symptoms that are commonly reported by the patients taking Zotiraciclib (TG02). It takes 5-10 minutes to complete the questionnaire. It will only be completed by the patient, unless changes in vision or weakness make this difficult. If this occurs, then the caregiver may read the questions to the patient or assist with marking the answers as described by the patient. A patient caregiver may complete the questionnaires as a patient-preference proxy if the patient's deficits preclude self-report. The questionnaires are allowed to be completed within +/-2 days form the scheduled dates. The PRO-CTCAE is only going to be conducted in the cohort expansion part of the study.
Patients will complete at least 2 questionnaires per cycle.

*The PRO-CTCAE will not be performed in the Post Interim Analysis cohort.

3.5 ASSESSMENTS WHILE ON STUDY THERAPY

3.5.1 Cycle 1 Assessments

During the 2nd week of treatment (i.e. 28-day cycle), the patient will have a complete blood count (CBC) with differential and platelet count. In phase I study, if grade 3 neutropenia is identified, repeat CBC with differential every 3 days until it improves to ⩽ grade 2. Patient may have CBC drawn at NIH lab or outside community lab.

During the 4th week of treatment (i.e. 28-day cycle), the patient will have the following assessments:

- Physical exam with vital signs, neurological exam, neurological function score and neurological examination (including documentation of the patient's Karnofsky Performance Status), mental status score. Weight is also included. Toxicity monitoring.
- A complete blood count (CBC) with differential and platelet count. In phase I study, if grade 3 neutropenia is identified, repeat CBC with differential every 3 days until it improves to ≤ grade 2.
- PT, PTT, INR, total protein, albumin, calcium, phosphorus, magnesium, glucose, BUN, creatinine, sodium, potassium, total bilirubin, alkaline phosphatase, LDH, SGPT (ALT), SGOT (AST) and a pregnancy test for women of childbearing potential
- 12-lead EKG.

On days 2, 8, 14, 21, and 28, patients will complete PRO-CTCAE form. (+/- 2 days).

The PRO-CTCAE will not be performed in the Post Interim Analysis cohort.

3.5.2 Cycle 2 Assessments

During the 2nd week of treatment, the patient will have a complete blood count (CBC) with differential and platelet count. In phase I study, if grade 3 neutropenia is identified, repeat CBC with differential every 3 days until it improves to ≤ grade 2. Patient may have CBC drawn at NIH lab or outside community lab.

During the 4th week of treatment (i.e. 28-day cycle), the patient will have the following assessments:

- Physical exam with vital signs, neurological exam, neurological function score and neurological examination (including documentation of the patient's Karnofsky Performance Status), mental status score. Weight is also included.
- A Gd-DPTA enhanced MRI, with DWI, ADC, SWI, FLAIR, perfusion, dynamic contrast enhanced and dynamic susceptibility contrast sequences should be performed.
- MD Anderson Symptom Inventory-Brain Tumor Module (MDASI-BT). If the MDASI-BT is completed by the patient for Natural History study within 14 days from the MRI assessment, it can be used but only if it was administered prior to informing patient of tumor imaging results.
- Toxicity monitoring

- A complete blood count (CBC) with differential and platelet count every two weeks. In phase I study, if grade 3 neutropenia is identified, repeat CBC with differential every 3 days until it improves to ⩽ grade 2.
- PT, PTT, INR, total protein, albumin, calcium, phosphorus, magnesium, glucose, BUN, creatinine, sodium, potassium, total bilirubin, alkaline phosphatase, LDH, SGPT (ALT), SGOT (AST) and a pregnancy test for women of childbearing potential
- 12-lead EKG.
- Response evaluation.

3.5.3 Subsequent cycles

For all subsequent odd-numbered cycles (i.e. cycle 3, 5, 7, 9, 11), patients will repeat assessments outlined in section **3.5.1**

For all subsequent even-numbered cycles (i.e. cycle 4, 6, 8, 10, 12), patients will repeat assessments outlined section **3.5.2**.

3.6 ASSESSMENTS AFTER COMPLETION OF STUDY THERAPY

Follow-Up Phase – Survival/Post-Disease Progression

Once a subject completes Phase II of study therapy or starts a new anti-cancer therapy, the subject moves into the survival follow up phase and should be contacted approximately within every 6 months to assess for survival status until death, withdrawal of consent, or the end of the study, whichever occurs first.

3.7 CCR SELF-ADMINISTERED STUDY DRUGS POLICY

All oral self-administered investigational agents will be properly accounted for, handled, and disposed in accordance with existing federal regulations and principles of Good Clinical Practice. All oral study drugs will be recorded in the patient diaries found in Appendix **15.7.** This will be used as a memory aide for subjects. A clinical research team maintains the primary source record.

3.8 STUDY CALENDAR

	Screening/ Baseline	Cycle 1				Cycle 2				Subsequent Odd numbered cycles		Subsequent Even numbered cycles		Off-study visit[8]
Procedure		**Wk 1**	**Wk 2***	**Wk 3**	**Wk 4***	**Wk 1**	**Wk 2***	**Wk 3**	**Wk 4***	**Wk 2***	**Wk 4***	**Wk 2***	**Wk 4***	**30 days (+/- 15) after last dose of treatment [8]**
Informed Consent	X													
Medical History	X													
Physical Exam[1]	X				X[1]				X[1]		X[1]		X[1]	X[1]
MDASI-BT	X								X				X[7]	
ProCTCAE***	X	X	X	X	X	X	X	X	X	X	X	X	X	
GD-DPTA MRI of brain	X								X				X[6]	
Hematology[2]	X		X		X		X		X	X	X	X	X	X
Chemistry[3]	X				X				X		X		X	X
PT/PTT/INR	X				X				X		X		X	
12-lead EKG	X				X				X				X	
Pregnancy Test [4]	X				X				X		X		X	X
Tumor Pathology	X													
Paraffin Tissue block [5]	X													
Response evaluation									X				X	
Research samples (PK, PG, neutrophil)[9,10]		X[9,10]												

1. Physical examination includes vital signs, neurological exam, neurological function score, Karnofsky performance score, mental status score. Height and weight are also included. **Height measured only at baseline.**
2. Includes CBC with differential and platelets. In phase I study, once grade 3 neutropenia is identified, CBC with differential will be tested every 3 days until it improves to ⩽ grade 2.
3. Includes: Total protein, albumin, Ca, Mg, P, glucose, BUN, creatinine, Na, K, total bilirubin, alkaline phosphatase, LDH, SGPT (ALT), SGOT (AST).
4. Only for women of childbearing potential as defined in section **15.2.** To be performed before each new cycle of therapy.
5. If a tissue block is unavailable, unstained slides are acceptable.
6. Brain MRI w/wo contrast needs to be done every two cycles of treatment.
7. MDASI-BT questionnaire needs to be completed at imaging cycles within +/- 1week. (prior to imaging results having been discussed with the patient). If the MDASI-BT is completed by the patient for Natural History study (CC 16C0151) within 14 days from the MRI assessment, it can be used but only if it was administered prior to informing patient of tumor imaging results.
8. If patient does not return for 30-day safety visit, patient will be taken off study for reason listed in section 3.10.3 If patient does not return, physical exam and blood work is not mandatory.
9. On cycle 1 day -3, collect following samples from patients in the **Phase 1 cohort expansion only**: PK studies (1 6mL green sodium heparin tube at following timepoints-pre-dose, 1hr, 2hr, 4hr, 12hr, 24hr, 48hr, and 72hr post-oral dose); PG studies (1 purple tube EDTA, 6mL, pre-dose) and neutrophil analysis (2 additional purple top EDTA, 6mL each for CBC/diff and neutrophil marker; and 1 additional heparinized green top tube, 6 mL for plasma isolation for monitoring neutrophil activation markers) at following timepoints- pre-dose, 1hr, 2hr, 4hr, 12hr, 24hr, 48hr, and 72hr post-oral dose). All effort will be made to collect samples at prescribed time points; missed timepoints or collections outside the window will be documented but not constitute a deviation.
10. Research sample will be collected for PK/PG study from patients enrolled to the **Post-Interim Analysis cohort** at the following timepoints: pre-dose, 1hr, 2hr, 4hr, 24hr, and 48hr post-oral dose. This will avoid the requirement for inpatient admission. The neutrophil sample will not be collected.

*+/- 3 business days

** Allowance for scheduling changes: Brief interruption and delay in the 28-day cycle may occasionally be required due to travel delays, airport closure, inclement weather, family responsibilities, security alerts and government holidays, etc. This can also extend to complications of disease not attributable to disease progression or protocol therapy. These delays will not be considered protocol deviation. However, any interruption more than 14 calendar days will not be permitted.

*** on days 2, 8, 14, 21, and 28 (+/- 2 days). The ProCTCAE data will not be collected from patients enrolled to the post-interim analysis cohort.

3.9 SURGICAL GUIDELINES

If neurosurgical management is required for reasons not due to tumor progression, these procedures must be documented, including the indications for surgery, the surgical operative note and pathology report. Study treatment may be resumed per discretion of the PI.

3.10 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

3.10.1 Criteria for Removal from Protocol Therapy

- After administration of 12 cycles of treatment, unless there is clear evidence of clinical benefit to justify continuation of treatment based on treating physician's opinion.
- Progressive disease if patient not eligible for or refuses crossover or progressive disease * after crossover to TMZ+ Zotiraciclib (TG02)
- The patient may withdraw from the study at any time for any reason
- Unacceptable Toxicity as defined in section **3.3**
- Investigator discretion
- Treatment related adverse events not resolved within a 4-weeks rest period or requiring more than two dose de-escalations of any drug whichever is earlier.
- Pregnancy

* Delayed imaging responses have been observed in some patients who received protocol treatment. If a patient is clinically stable or clinically improved, even while MRI showed >25% increase in tumor size, the patient may continue on study treatment per P.I. discretion for one additional cycle, at which time a repeat scan will be done. If tumor size continues to increase, disease progression will be dated back to the date of prior MRI.

3.10.2 Off-Study Visit

Prior to removal from study, effort must be made to have all subjects complete a safety visit approximately 30 days following the last dose of study therapy.

If patient does not return for off study follow up safety visit, patient will be taken off study per appropriate reason listed in section **3.10.3.**

During the follow up safety visit, the following study tasks will be performed:

- A complete physical (including baseline blood pressure measurement) and neurological examination (include documentation of the patients' Karnofsky Performance Status).
- MD Anderson Symptom Inventory-Brain Tumor Module (MDASI-BT) **(Error! Reference source not found.**)
- Laboratory tests will include CBC, differential, platelets, total protein, albumin, calcium, phosphorus, magnesium, glucose, BUN, creatinine, sodium, potassium, total bilirubin, alkaline phosphatase, LDH, SGPT (ALT), SGOT (AST) and a pregnancy test for women of childbearing potential.
- Take patient off study (section **Error! Reference source not found.**)

3.10.3 Off-Study Criteria

- Participant requests to be withdrawn from study
- Patients lost to or refuse to follow up

- Death
- Screen failure
- PI discretion based on clinical indications
- Patient to start alternative therapy
- Patient completed follow up period
- Phase II patient completed follow up until death

4 CONCOMITANT MEDICATIONS/MEASURES

4.1 MEDICATIONS TO AVOID OR USE WITH PRECAUTION (ZOTIRACICLIB [TG02])

4.1.1 Medications that Prolong QTc

Use of certain medications that prolong QTc interval should be avoided throughout the trial. These medications include but are not limited to: azithromycin, clarithromycin, erythromycin, roxithromycin, metronidazole (with alcohol), moxifloxacin, fluconazole (in cirrhosis), ketoconazole, nelfinavir, chloroquine, mefloquine, halothane, disopyramide, procainamide, quinidine, amiodarone, sotalol, amitriptyline, clomipramine, impipramine, dothiepin, doxepin, risperidone, fluphenazine, haloperidol, clozapine, thioridiazine, ziprasidone, pimozide, droperidol, terfenadine, astemizole, probucol, dolasetron mesylate, and cisapride.

However, it is understood that patients with malignancy treated with potentially myelotoxic cytotoxic chemotherapy may require certain classes of medications such as azole antifungal agents and certain antibiotics that have been reported to prolong QTc. If patients require these medications every effort should be made to maintain a normal electrolyte balance with special attention to K^+ and Mg^{++} levels. An ECG in triplicate should be obtained after beginning such a medication to determine any change to the QTc duration based on the average QTc of triplicate ECGs.

In the event of QT/QTc prolongation, electrolytes will be corrected to keep the potassium and magnesium within normal limits and the ECG in triplicate will be repeated. Patients may be monitored on telemetry at the Investigator's discretion.

During the trial, patients who show a QTc interval prolongation as per CTCAE (based on the average QTc of triplicate ECGs) as calculated by Fridericia's correction formula, should be tested for the electrolytes noted above. If the electrolytes are at normal levels but QTc interval remains prolonged, study drug should be held until QTc interval is no longer prolonged.

4.1.2 CYP450 Drug Interactions (Related to Drug Metabolism)

Strong inhibitors/inducers of CYP3A4 and CYP1A2 and drugs that are known substrates for CYP1A2 and CYP2D6 with a narrow therapeutic index should be used with caution in this study.

Known inhibitors of CYP1A2 include acyclovir and fluvoxamine, and known inhibitors of CYP3A4 include atazanavir, clarithromycin, indinavir, itraconazole, ketoconazole, nefazodone,

nelfinavir, ritonavir, saquinavir, and telithromycin.

Known inducers of CYP1A2 include omeprazole, insulin, and cigarette smoking, and known inducers of CYP3A4 include rifampin and carbamazepine.

Sensitive CYP1A2 substrates with narrow therapeutic indices include theophylline and tizanidine, and sensitive CYP2D6 substrates with narrow therapeutic indices include thioridazine and tamoxifen.

Exceptions are allowed per PI discretion, if clinically indicated.

4.2 MEDICATIONS TO AVOID OR USE WITH PRECAUTION (TMZ)

Administration of valproic acid decreases oral clearance of temozolomide by about 5%.

4.3 G-CSF AND ERYTHROPOIETIN ADMINISTRATION:

Routine prophylactic use is not permitted. However, therapeutic use in patients with complications (severe neutropenia with fever or anemia), may be considered at the investigator's discretion.

4.4 PLATELET SUPPORT:

Prophylactic use of platelet is only used when Platelet count < 20 x 10^9/L. Platelet transfusion is provided for active bleeding patients with thrombocytopenia to keep platelet count above 50 x 10^9/L.

4.5 CORTICOSTEROIDS

Should be used in the smallest dose to control symptoms of cerebral edema and mass effect and discontinued if possible.

4.6 FEBRILE NEUTROPENIA

It may be managed according to the local institution's Infectious Disease guidelines. Measures may include appropriate laboratory testing, including blood and urine cultures and the institution of broad-spectrum antibiotics. If a source for the fever is not identified or the fever resolves when the neutrophil count recovers, antibiotics may be discontinued, and the patient observed.

4.7 ANTI-EMETICS

Prophylactic and therapeutic use is allowed. Other concomitant medications and the therapies considered necessary for the well-being of the patient may be given at the discretion of the treating physician. All concomitant medications must be recorded.

4.8 OTHER ANTICANCER OR EXPERIMENTAL THERAPIES

No other anticancer therapy (including chemotherapy, radiation, hormonal treatment or immunotherapy) of any kind is permitted during the study period. No other antitumor drugs under investigation may be used concomitantly with the study drug.

5 BIOSPECIMEN COLLECTION

5.1 CORRELATIVE STUDIES FOR RESEARCH

Test/assay	Volume blood (approx.)	Type of tube	Collection point (+/- 48hrs)	Location of specimen analysis
Target DNA Sequencing	Tumor tissue block	-	Initial and/or Recurrence	Laboratory of Pathology
Western Blot and PCR	Fresh Frozen Tumor	-	Surgery at Disease recurrence	NOB Laboratory
PK and PG*	6mL	Sodium Heparin (green top)	Cycle 1 day -3 at the following time points: pre-dose, 1hr, 2hr (peak), 4hr, 12hr-, 24hr, 48hr, and 72hr post oral dose. For patients in the cohort expansion only	Dr. Figg's Lab
	Up to 6mL	EDTA (purple top)	Cycle 1 day – 3 For patients in the cohort expansion only	Dr Figg's Lab
Neutrophil analysis**	12mL	EDTA (purple top)	Cycle 1 day -3 at the following time points: pre-dose, 1hr, 2hr (peak), 4hr, 12hr, 24hr, 48hr, and 72hr post oral dose. For patients in the cohort expansion only	(1 tube) Processed by NOB Laboratory and sent to Dept of Laboratory Medicine
				(1 tube) Processed by NOB Laboratory and sent to Neutrophil Monitoring Lab (NML)

Test/assay	Volume blood (approx.)	Type of tube	Collection point (+/- 48hrs)	Location of specimen analysis
	6mL	(green top)	Cycle 1 day -3 at the following time points: pre-dose, 1hr, 2hr (peak), 4hr, 12hr, 24hr, 48hr, and 72hr post oral dose. For patients in the cohort expansion only	Processed by NOB Laboratory and sent to Neutrophil Monitoring Lab (NML)
*PK/PG analysis: For patients in Post-Interim Analysis cohort, samples will be collected in Cycle 1 day -3 at the following time points only: pre-dose, 1hr, 2hr (peak), 4hr, 24hr, and 48hr post oral dose. **Neutrophil analysis will not be collected and performed in patients in Post-Interim Analysis cohort.				

5.1.1 The brain tumor tissue block from initial and /or recurrence will be collected to detect MGMT methylation, *TP53, PTEN, IDH* mutation, *MYC* amplification status by performing targeted DNA sequencing at the laboratory of Pathology, CCR, NIH (if not included in the Natural History Study [CC 16C0151]).

5.1.1.1 Tissue blocks can be shipped to Department of Pathology at NCI at the following address:

Laboratory of Pathology
Center for Cancer Research
National Cancer Institute
Building 10, Room 2S235
Bethesda, MD 20892-1500
Ph: 301-480-5010

5.1.1.2 Procedures for storage of tissue specimens in the Laboratory of Pathology

Tissues designated for clinical diagnostics are transported to the Laboratory of Pathology (LP) where they are examined grossly, and relevant portions are fixed, embedded in paraffin and sectioned and stained for diagnostic interpretation. Unutilized excess tissues are stored for up to three months, in accordance with College of American Pathologists/Joint Commission on Accreditation of Healthcare Organizations (CAP/JCAHO) guidelines, and then discarded. Following completion of the diagnostic workup, the slides and tissue blocks are stored indefinitely in the LP's clinical archives. All specimens are catalogued and retrieved utilizing the clinical laboratory information systems, in accordance with CAP/JCAHO regulations. The use of any stored specimens for research purposes is only allowed when the appropriate IRB approval has been obtained. In some cases, this approval has been obtained via the original protocol on which the patient was enrolled.

5.1.2 Fresh frozen brain tumor tissue will be obtained if patient undergoes surgery at the time of disease progression. Pro-survival proteins, including Mcl-1, XIAP and survivin will be tested at protein by Western blot and at mRNA level by PCR in the NOB laboratory.

5.1.2.1 The samples should be shipped to the following address ON DRY ICE in appropriately labeled package in accordance with local and federal regulations for biological material:

Wei Zhang MD, PhD.
Neuro-Oncology Branch, NCI, NIH
Bldg. 37, Room 1142
Bethesda, MD 20892
Office: 240-760-6835 or 301-435-3136
Cell: 240-274-0469
Pager: 102-12209

5.1.2.2 If the surgery is performed at Neurosurgery Department at NINDS, NIH, the following personnel will be contacted for tissue pick up.

Wei Zhang MD, PhD.
Neuro-Oncology Branch, NCI, NIH
Bldg. 37, Room 1142
Bethesda, MD 20892
Office: 240-760-6835 or 301-435-3136
Cell: 240-274-0469
Pager: 102-12209

5.1.3 Pharmacokinetic (PK) and Pharmacogenetic (PG)Studies

5.1.3.1 Pharmacokinetic Studies

PK studies will be done in all patients that are enrolled in the cohort expansion part and the Post-Interim Analysis cohort. Blood samples for the determination of plasma levels of Zotiraciclib (TG02) will be obtained from each patient (see section **5.1.3.3** for schedule and tube types). Bioanalytical measurements for Zotiraciclib (TG02) will be conducted on an ultra HPLC-MSMS system using an assay developed and validated by the Clinical Pharmacology Program. This data will be used to monitor plasma concentrations of Zotiraciclib (TG02) to correlate to pharmacodynamic endpoints, clinical response, toxicity, and pharmacogenetic analyses.

5.1.3.2 Pharmacogenetic studies (PG)

PG studies will be done in all patients that are enrolled in the cohort expansion part and the Post-Interim Analysis cohort. One blood sample per patient (6mL) will be collected (see section **5.1.3.3** for schedule) to analyze the genomic DNA and assess genotype of the most relevant drug metabolizing enzymes and transporters (DMET). DNA will be analyzed on a DMET Plus (Affymatrix) genotyping platform that has the capacity to test for 1,936 genetic variations in 225 drug disposition genes, including 47 CYP (phase I metabolism) genes, 13 non-CYP (phase I metabolism) genes, 78 phase II metabolizing genes (including UGTs), 63 transporters, 4 genes involved in facilitation of drug transporters, 9 genes involved in global regulation of drug metabolizing/transporting proteins, 4 drug binding proteins.

Of specific interest to Zotiraciclib (TG02) are polymorphisms in CYP3A4, CYP1A2, and ABCB1 (P-glycoprotein), all of which are included in the DMET analysis.

The samples will be placed at room temperature. The date and exact time of each blood draw should be recorded on the sample tube and the PK/PG sheet. Contact Dr. Figg's Clinical Pharmacology Program (Blood Processing Core) in Bldg. 10/5A09 at NCIBloodcore@mail.nih.gov at least 24 hours before transporting samples (the Friday before is preferred).

5.1.3.3 Sample Collection

PKs: Blood samples for the determination of plasma levels of Zotiraciclib (TG02) will be obtained from each patient via 6mL green sodium heparin tube collected on the first daily dose on cycle 1 day -3 at the following time points: pre-dose, 1hr, 2hr (peak), 4hr, 12hr, 24hr, 48hr, and 72hr post oral dose. For patients in the post-interim analysis cohort, blood will be collected, pre-dose, 1hr, 2hr, 4hr, 24hr, and 48hr post oral dose.

PGs: 6 mL of blood will be collected in an EDTA (purple top) tube on day -3 of cycle 1.

5.1.3.4 Sample processing

The samples will be placed immediately on wet ice and refrigerated. The date and exact time of each blood draw should be recorded on the sample tube and the PK sheet.

Please e-mail NCIBloodcore@mail.nih.gov at least 24 hours before transporting samples (the Friday before is preferred).

For sample pickup, page 102-11964.

For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).

For questions regarding sample processing, contact NCIBloodcore@mail.nih.gov.

Upon arrival in the Clinical Pharmacology Program, samples will be centrifuged, and the plasma transferred into cryovials for storage at -80 C until the time of analysis. In addition, samples will be barcoded.

5.1.4 Neutrophil analysis

Neutrophil analysis studies will be done in all patients that are enrolled in the cohort expansion part.

Three tubes of blood will be collected at all PK time points for the following studies: WBC and differential analysis, neutrophil morphology studies (photographs), neutrophil function assays, surface marker analysis and for spinning out cells and saving plasma to send to Dr. Douglas Kuhns for monitoring activation markers released into the plasma such as specific granule marker. (See section **5.1.4.1** for tube types and collection information. Note: The pediatric size tubes can be used as alternatives for blood sample collection, if it is necessary.)

WBC with differential and imaging analysis will be done at Department of Laboratory Medicine. Plasma and neutrophil isolation will be done by NOB laboratory and analysis will be done in Neutrophil Monitoring Laboratory (NML).

5.1.4.1 Collection

a. CBC with differential (a tube of 6 cc blood collected in purple top tube) will be done on cycle 1 day -3 at the following time points*: pre-dose, 1hr, 2hr, 4hr, 12hr, 24hr, 48hr, and 72hr post oral Zotiraciclib (TG02). Neutrophil morphology will be studies by using Cellavision program.
b. Collect blood sample (6 cc of blood in heparinized green top tube) at the time of PK sample collection for plasma isolation for monitoring neutrophil activation markers.
c. Collect (6 cc of blood in purple top tube with EDTA), fix and stain neutrophils at the same time points of the PK sample collection. Samples will be used for neutrophil cell surface markers including but not limited to CD11a, CD11b, CD11c, CD32, CD18, CD67, CD64, CD18, CD45, CD62L, CD63, 7D5.

* All effort will be made to collect samples at prescribed time points; missed timepoints or collections outside the window (+/- 30 minutes from the scheduled time) will be documented but not constitute a deviation.

5.1.4.2 Contact of NOB laboratory

Ying Pang, MD., PhD.
Neuro-Oncology Branch, NCI, CCR
Bldg. 37, Room 1142
Bethesda, MD 20892
Office: 240-760-6216

5.1.4.3 Contact of NML

Douglas Kuhns, PhD.
Building 310, Rm 204
Frederick, MD 21702
Office: 301-846-6378

5.1.5 Labeling of Samples

Labels on all samples will at least include the following information:

- Patient initials, ID number
- Protocol number
- Collection date

5.2 SAMPLE STORAGE, TRACKING AND DISPOSITION

Samples will be ordered in CRIS and tracked through a Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed.

Samples will not be sent outside NIH without appropriate approvals and/or agreements, if required.

5.2.1 Clinical Pharmacology Program

All samples sent to the Clinical Pharmacology Program (CPP) will be barcoded, with data entered and stored in Labmatrix utilized by the CPP. This is a secure program, with access to Labmatrix limited to defined Figg lab personnel, who are issued individual user accounts. Installation of Labmatrix is limited to computers specified by Dr. Figg. These computers all have a password restricted login screen.

Labmatrix creates a unique barcode ID for every sample and sample box, which cannot be traced back to patients without Labmatrix access. The data recorded for each sample includes the patient ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location. Patient demographics associated with the clinical center patient number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).Barcoded samples are stored in barcoded boxes in a locked freezer at either -20 or -80°C according to stability requirements. These freezers are located onsite in the CPP and offsite at NCI Frederick Central Repository Services in Frederick, MD. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.

Access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in Labmatrix. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB approved protocol) and that any unused samples must be returned to the CPP. It is the responsibility of the NCI Principal Investigator to ensure that the samples requested are being used in a manner consistent with IRB approval.

Following completion of this study, samples will remain in storage as detailed above. Access to these samples will only be granted following OHSRP/IRB approval of an additional protocol, granting the rights to use the material or if the use is not considered to be human subjects research.

5.2.2 Neutrophil Monitoring Laboratory (NML) / Frederick Laboratory

Plasma and neutrophil isolation will be done by NOB laboratory and analysis will be done in Neutrophil Monitoring Laboratory (NML) in Frederick.

Samples will be sent to the NOB laboratory and sent to Frederick laboratory will be barcoded with data entered and stored in the Labmatrix system utilized by the CCR, NCI. This is a secure system with access limited to defined personnel. All such personnel with access to patient information annually complete the NIH online Protection of Human Subjects course.

Labmatrix creates a unique barcode ID for every sample which cannot be traced back to patients without Labmatrix access. The data recorded for each sample includes the patient ID, name, trial name/protocol number, date/time drawn, as well as box and freezer location. Patient demographics associated with the Clinical Center patient number are provided in the system. For

each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).

5.2.3 Department of Laboratory Medicine

Samples will be processed by the NOB laboratory and then sent to Department of Laboratory Medicine will be barcoded with data entered and stored in the Labmatrix system utilized by the CCR, NCI. This is a secure system with access limited to defined personnel. All such personnel with access to patient information annually complete the NIH online Protection of Human Subjects course.

5.2.4 Protocol Completion/Sample Destruction

All specimens obtained in the protocol are used as defined in the protocol. Any specimens that are remaining at the completion of the protocol will be stored in the conditions described below. The study will remain open so long as sample or data analysis continues. Samples from consenting subjects will be stored until they are no longer of scientific value or if a subject withdraws consent for their continued use, at which time they will be destroyed.

If the patient withdraws consent the participant's data will be excluded from future distributions, but data that have already been distributed for approved research use will not be able to be retrieved.

The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of section 7.2.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system (C3D) and ensuring data accuracy, consistency and timeliness. The MDASI-BT responses will be entered by study subjects directly into Scribe/Labmatrix.
The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. All data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

All adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event.

Document AEs from the first study intervention, Study Day 1, through 30 days after the last administration of investigational agent/intervention. Adverse events that are serious need to be recorded through 30 days after the last administration of investigational agent/intervention. Beyond 30 days after the last intervention, only adverse events which are serious and related to the study intervention need to be recorded.

An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

End of study procedures: Data will be stored according to HHS, FDA regulations and NIH Intramural Records Retention Schedule as applicable.

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, this will be reported expeditiously per requirements in section **7.2.1**.

6.1.1 Non-Relevant Toxicities for Brain Tumor Protocols

Lymphopenia is a common finding among patients with primary brain tumors and is directly attributable to concurrent use of corticosteroids. To date, even Grade III lymphopenia has not been associated with a worsening of clinical outcome. Therefore, we will not consider this parameter in the determination of optimal dosing of drugs.

Weight gain is a common finding among patients with primary brain tumors. This too is directly attributed to the concurrent use of corticosteroids. Doses of steroids or study agents will not be modified as a consequence of weight gain. Corticosteroid dosage will be based on maintenance of control of edema in the brain and the standard clinical practice to use the minimal effective dose of corticosteroids will be employed.

Alopecia is a common occurrence in patients with brain tumors as a consequence of cranial radiotherapy. Alopecia will not be recorded or graded as toxicity.

Venous thromboembolic disease is a common complication occurring in up to 30% of patients with malignant gliomas.[47, 48] In this study, patients who develop deep vein thrombosis may receive anticoagulation and resume therapy once they are stable on anticoagulation. Grade III deep vein thrombosis will not be considered a DLT for the phase I portion.

The routine use of filgrastim or other white cell growth factors is not permitted during cycle 1 of the Phase I portion unless clinically indicated, but it may be used beyond this period or once the assessment of DLT has been completed.

6.2 DATA SHARING PLANS

6.2.1 Human Data Sharing Plan

What data will be shared?

I will share human data generated in this research for future research as follows:

- Coded, linked data in an NIH-funded or approved public repository.
- Coded, linked data in BTRIS (automatic for activities in the Clinical Center)
- Coded, linked or identified data with approved outside collaborators under appropriate agreements.

How and where will the data be shared?

Data will be shared through:

- BTRIS (automatic for activities in the Clinical Center)
- Approved outside collaborators under appropriate individual agreements.
- Publication and/or public presentations.

When will the data be shared?

- Before publication.
- At the time of publication or shortly thereafter.

6.2.2 Genomic Data Sharing

Unlinked genomic data will be deposited in public genomic databases such as dbGaP in compliance with the NIH Genomic Data Sharing Policy.

6.3 RESPONSE CRITERIA

6.3.1 General

The primary efficacy endpoint for this study is progression free survival (PFS) from patient registration. However, objective response status should be measured and recorded.

6.3.2 Definitions

Evaluable for maximum tolerated dose: All subjects enrolled in the Phase I dose escalation cohorts from the time of their first treatment with Zotiraciclib (TG02) +TMZ to the end of cycle 1. Patients who develop a DLT during cycle 1 and thus do not complete treatment for the cycle should be considered evaluable for MTD.

Evaluable for toxicity: All eligible patients who complete at least one cycle of protocol therapy or who have a DLT after the protocol therapy is started are evaluable for toxicity from the time of their first treatment.

*If the patients miss doses of Zotiraciclib (TG02) or TMZ in the first cycle of treatment due to reasons that are unrelated to toxicity, it will be the PI's decision about whether they are evaluable for toxicity in the dose finding stage.

Evaluable for objective response: Only those patients who have measurable disease present at baseline, have received at least one cycle of therapy at MTD and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified 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.)

Phase I patients removed from study treatment within 28 days for reasons other than toxicity may be replaced.

6.3.3 Disease Parameters

Measurable Disease: Bi-dimensionally measurable lesions with clearly defined margins by GD-DPTA enhanced MRI scan.

Evaluable Disease: Uni-dimensionally measurable lesions, masses with margins not clearly defined. Patients with only this kind of imaging will not be allowed to enter this study unless they have recently undergone surgery and have histologically proven recurrent disease.

Non-Evaluable Disease: Not Applicable for response evaluation.

Objective-Status, To Be Recorded at Each Evaluation: If there are too many measurable lesions to measure at each evaluation, choose the largest two to be followed before a patient is entered on study. The remaining lesions will be considered evaluable for the purpose of objective status determination. Unless progression is observed, objective status can only be determined when ALL measurable and evaluable sites and lesions are assessed.

6.3.4 Response Criteria

Response and progression will be evaluated in this study using the new proposed criteria Response Assessment in Neuro-Oncology Criteria (RANO) (see Appendix **15.5**).[49]

Complete response: Requires all of the following:

- Complete disappearance of all enhancing measurable and non-measurable disease sustained for at least 4 weeks.
- No new lesions and stable or improved non-enhancing lesions (T2/FLAIR)
- Patient off steroids or on physiologic replacement only.
- Stable or improved clinically.

Partial response: Requires all of following:

- ≥ 50 % decrease compared to baseline (prior to treatment) in the sum of the products of perpendicular diameters of all measurable enhancing lesions sustained for at least 4 weeks;
- No new lesions and stable or improved non-enhancing lesions (T2/FLAIR).
- Patients off steroids or on physiologic replacement only.

- Stable or improved clinically.

Stable disease: Requires all of the following:

- Not complete response, partial response or progression.
- Stable non-enhancing lesions (T2/FLAIR).
- Patient on same or lower dose of steroids compared to baseline scan.

Progression: Requires any of the following:

- ≥ 25 % increase compared to baseline in the sum of the products of perpendicular diameters of enhancing lesions compared with the smallest measurement obtained either at baseline or best response with the patient on stable or increasing doses of steroids.
- Significant increase in T2/FLAIR non-enhancing lesions with the patient on stable or increasing doses of steroids (not caused by comorbid events e.g. Radiation therapy, demyelination, ischemic injury, infection, seizures, medication adverse events or changes in corticosteroid dose).
- Any new lesions.
- Failure to return for evaluation as a result of death or deteriorating condition or clear progression of non-measurable disease.

6.3.5 Patients will undergo response evaluations every 2 months.

- RANO criteria will be used to determine response and progression (includes CR, PR, SD and PD) **15.5.**
- Neurological exam.
- Performance status by Karnofsky Performance Status.

6.3.6 Evaluation of Best Overall Response

This will be calculated from the sequence of objective statuses. For patients with all disease sites assessed every evaluation period, the best response will be defined as the best objective status as measured according to Section **6.3.4**. If the response does not persist at the next regular scheduled MRI, the response will still be recorded based on the prior scan but will be designated as a non-sustained response. If the response is sustained, e. g. still present on the subsequent MRI, it will be recorded as a sustained response, lasting until the time of tumor progression. Best response is unknown if the patient does not qualify for a best response or increasing disease and if all objective status determinations before progression are unknown.

6.3.7 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

6.3.8 Progression-Free Survival

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

6.3.9 Time to Death:

From date of registration to date of death due to any cause.

6.3.10 Neurological Exam:

Although not used for determining response, it is useful to evaluate improvement in the neurologic exam, (as compared to the baseline assessment), that should coincide with objective measurement of tumor size. The criteria are as follows:

0= No neurologic symptoms, fully active at home/work without assistance

1= Minor neurologic symptoms, fully active at home/work without assistance

2= Moderate neurologic symptoms, fully active at home/work but requires assistance

3= Moderate neurologic symptoms, less than fully active at home/work and requires assistance

4= Severe neurologic symptoms, totally inactive requiring complete assistance at home or institution, unable to work

6.4 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40).

7 NIH REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found here.

7.2 OHSRP OFFICE OF COMPLIANCE AND TRAINING/IRB REPORTING

7.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found here. Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported per these policies.

7.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found here.

7.3 NCI CLINICAL DIRECTOR REPORTING

Problems expeditiously reported to the OHSRP in iRIS will also be reported to the NCI Clinical Director. A separate submission is not necessary as reports in iRIS will be available to the Clinical Director.

In addition to those reports, all deaths that occur within 30 days after receiving a research intervention should be reported via email to the Clinical Director unless they are due to progressive disease.

To report these deaths, please send an email describing the circumstances of the death to Dr. Dahut at NCICCRQA@mail.nih.gov within one business day of learning of the death.

7.4 NIH REQUIRED DATA AND SAFETY MONITORING PLAN

7.4.1 Principal Investigator/Research Team

The clinical research team will meet on a regular basis when patients are being actively treated on the trial to discuss each patient. Decisions about dose level enrollment and dose escalation if applicable will be made based on the toxicity data from prior patients.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Events meeting requirements for expedited reporting as described in section **7.2.1** will be submitted within the appropriate timelines.

The principal investigator will review adverse event and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

8 SPONSOR SAFETY REPORTING

8.1 DEFINITIONS

8.1.1 Adverse Event

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (ICH E6 (R2))

8.1.2 Serious Adverse Event (SAE)

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

- Death,
- A life-threatening adverse event (see section **8.1.3**)
- Inpatient hospitalization or prolongation of existing hospitalization
    - A hospitalization/admission that is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study), a planned hospitalization for pre-existing condition, or a procedure required by the protocol, without a serious deterioration in health, is not considered a serious adverse event.
    - A hospitalization/admission that is solely driven by non-medical reasons (e.g., hospitalization for patient convenience) is not considered a serious adverse event.
    - Emergency room visits or stays in observation units that do not result in admission to the hospital would not be considered a serious adverse event. The reason for seeking medical care should be evaluated for meeting one of the other serious criteria.
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.1.3 Life-threatening

An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death. (21CFR312.32)

8.1.4 Severity

The severity of each Adverse Event will be assessed utilizing the CTCAE version 4.0.

8.1.5 Relationship to Study Product

All AEs will have their relationship to study product assessed using the terms: related or not related.

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.

- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

8.2 ASSESSMENT OF SAFETY EVENTS

AE information collected will include event description, date of onset, assessment of severity and relationship to study product and alternate etiology (if not related to study product), date of resolution of the event, seriousness and outcome. The assessment of severity and relationship to the study product will be done only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as the site principal investigator or sub-investigator. AEs occurring during the collection and reporting period will be documented appropriately regardless of relationship. AEs will be followed through resolution.

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.
- Recorded on the appropriate SAE report form, the medical record and captured in the clinical database.
- Followed through resolution by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

For timeframe of recording adverse events, please refer to section **6.1**. All serious adverse events recorded from the time of first investigational product administration must be reported to the sponsor**.**

8.3 REPORTING OF SERIOUS ADVERSE EVENTS

Any AE that meets protocol-defined serious criteria or meets the definition of Adverse Event of Special Interest that require expedited reporting must be submitted immediately (within 24 hours of awareness) to OSRO Safety using the CCR SAE report form.

All SAE reporting must include the elements described in section **8.2**.

SAE reports will be submitted to the Center for Cancer Research (CCR) at: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. CCR SAE report form and instructions can be found at: https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=157942842

Following the assessment of the SAE by OSRO, other supporting documentation of the event may be requested by the OSRO Safety and should be provided as soon as possible.

8.4 SAFETY REPORTING CRITERIA TO THE PHARMACEUTICAL COLLABORATORS

All events listed below must be reported in the defined timelines to OSROSafety@mail.nih.gov.

The CCR Office of Regulatory Affairs will send all reports to the manufacturer as described below:

Safety reports (subject to redaction, if required) sent to the FDA will be concurrently sent to the manufacturer:

Jillian Chapas-Reed

Vice President, Clinical Operations

Adastra Pharmaceuticals, Inc.

Telephone: 410-920-2475

jchapas-reed@adastrarx.com

www.adastrarx.com

8.5 REPORTING PREGNANCY

8.5.1 Maternal exposure

If a patient becomes pregnant during the course of the study, the study treatment should be discontinued immediately, and the pregnancy reported to the Sponsor no later than 24 hours of when the Investigator becomes aware of it. The Investigator should notify the Sponsor no later than 24 hours of when the outcome of the Pregnancy become known,

Pregnancy itself is not regarded as an SAE. However, congenital abnormalities or birth defects and spontaneous miscarriages that meet serious criteria (section **8.1.2**) should be reported as SAEs.

The outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) should be followed up and documented.

8.5.2 Paternal exposure

Male patients should refrain from fathering a child or donating sperm during the study and for 30 days after the last dose of Zotiraciclib (TG02)

Pregnancy of the patient's partner is not considered to be an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) occurring from the date of the first dose until 30 days after the last dose should, if possible, be followed up and documented.

8.6 REGULATORY REPORTING FOR STUDIES CONDUCTED UNDER CCR-SPONSORED IND

Following notification from the investigator, CCR, the IND sponsor, will report any suspected adverse reaction that is both serious and unexpected. CCR will report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study product and the adverse event. CCR will notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, in accordance to 21 CFR Part 312.32.

All serious events will be reported to the FDA at least annually in a summary format.

9 CLINICAL MONITORING PLAN

As a sponsor for clinical trials, FDA regulations require the CCR to maintain a monitoring program. The CCR's program allows for confirmation of: study data, specifically data that could

affect the interpretation of primary and secondary study endpoints; adherence to the protocol, regulations, ICH E6, and SOPs; and human subjects protection. This is done through independent verification of study data with source documentation focusing on:

- Informed consent process
- Eligibility confirmation
- Drug administration and accountability
- Adverse events monitoring
- Response assessment.

The monitoring program also extends to multi-site research when the CCR is the coordinating center.

This trial will be monitored by personnel employed by a CCR contractor. Monitors are qualified by training and experience to monitor the progress of clinical trials. Personnel monitoring this study will not be affiliated in any way with the trial conduct.

10 STATISTICAL CONSIDERATIONS

10.1 PHASE I COMPONENT:

We will employ the Bayesian Optimal Interval (BOIN) design [46] to find the MTD. The BOIN design is a novel Bayesian dose-finding method that optimizes patient ethics by minimizing the chance of exposing patients to sub-therapeutic and overly toxic doses. The BOIN design yields an average performance comparable to that of the continual reassessment method (CRM) in terms of selecting the MTD, but has a lower risk of assigning patients to sub-therapeutic or overly toxic doses (i.e., better patient ethics).

There are two dosing schedule treatment arms in the Phase I study: the combination treatment with Zotiraciclib (TG02) and dd TMZ; and the combination treatment of Zotiraciclib (TG02) with mn TMZ. Dose finding will be conducted independently in these two arms. For each arm, the target toxicity rate is 0.35 and the maximum sample size for dose finding is 24 patients. We will enroll and treat patients in cohorts of size 3. We consider four dose levels and the starting dose is level 0 (i.e., TMZ 125mg/m2 + 200mg/day Zotiraciclib (TG02)).

Table 12

Level	**Arm**	
	TMZ (mg/m2 x 7 days on/7 days off)	**TG 02 (mg/day on 1, 12,15,26 per 28 days cycle)***
-1	125	150
0 (starting dose)	125	200
I	125	250
II	125	300**
Level	**Arm**	
	TMZ (mg/m2 daily)	**TG 02 (mg/day on 1, 12,15,26 per 28 days cycle)***
-1	50	150
0 (starting dose)	50	200
I	50	250
II	50	300**

* The first dose of Zotiraciclib (TG02) will be given 3 days prior to Day1 of cycle 1. The subsequent doses for cycle 1 will be given on days 1, 12, 15, and 26. Zotiraciclib (TG02) will be given on days 1, 12, 15, and 26 every 28-day cycle in all the cycles starting cycle 2.

** One patient in MN arm developed severe toxicities which lead to ICU admission after one dose of Zotiraciclib (TG02) at 300mg. The etiology was not completely clear. However, due to the patient's safety concern, does level II of Zotiraciclib (TG02) was determined to be eliminated in both study arms by the investigator and pharmaceutical manufacture.

The BOIN trial design is described as follows:

1. Patients in the first cohort are treated at dose level 0.

2. To assign a dose to the next cohort of patients, we conduct dose escalation/de-escalation according to the rule displayed in Table **13**.

Table 13: Dose escalation/de-escalation rule for the BOIN design.

	The number of patients treated **at the current dose**					
Action	3	6	9	12	15	18
Escalate if # of patients who experienced DLT <=	0	1	2	3	4	4
De-escalate if # of patients who experienced DLT >=	2	3	4	6	7	8
Eliminate if # of patients who experienced DLTs >=	3	5	6	7	9	10

When using Table **13**, please note the following:

(a) "Eliminate" means that we eliminate the current and higher doses from the trial to prevent treating any future patients at these doses because they are overly toxic.

(b) When we eliminate a dose, we automatically de-escalate the dose to the next lower level. When the lowest dose is eliminated, we stop the trial for safety. In this case, no dose should be selected as the MTD.

(c) If none of the actions (i.e., escalation, de-escalation or elimination) is triggered, we treat the new patients at the current dose.

(d) If the current dose is the lowest dose and the rule indicates dose de-escalation, we will treat the new patients at the lowest dose unless the number of DLTs reaches the elimination boundary, at which point we will terminate the trial for safety.

(e) If the current dose is the highest dose and the rule indicates dose escalation, we will treat the new patients at the highest dose.

(f) If the number of patients treated at the current (or any) dose reaches 12, we will stop the trial early and select the MTD as described below.

3. Repeat step 2 until the maximum sample size of 24 is reached or the trial is stopped.

After the trial is completed, we select the MTD based on isotonic regression as specified previously. [46] Specifically, we select as the MTD the dose for which the isotonic estimate of the toxicity rate is closest to the target toxicity rate. If there are ties, we select the higher dose level when the isotonic estimate is lower than the target toxicity rate; and we select the lower dose level when the isotonic estimate is greater than the target toxicity rate.

After identifying the MTD for each of the TMZ dosing schedules in combination with Zotiraciclib (TG02) (i.e., 125mg/m2 TMZ on days 1-7 and 15-21 per cycle, and 50 mg/m2 TMZ daily), cohort expansion will be conducted independently at each of the MTDs until the total number of patients treated at each of the MTDs reaches 18 patients (including the patients who have been treated in the dose-finding part). The dosing schedule with a higher PFS at 4 months (PFS4) will be selected and used in phase II. If the difference between the PFS4 values from the two full cohorts treated at each of the MTDs is less than 10% of the average of the two PFS4 values, then the dosing schedule with fewer AEs will be selected as the "winner." During the cohort expansion phase, the elimination rule displayed in the last row of Table **13** will be used to monitor toxicity. Given the cohort expansion of 18 patients at the MTD, there is 72.6% chance to correctly pick the better schedule when the PFS4 for two schedules are 50% and 60% (i.e., 10% difference in PFS4), respectively; and there is 81.9% chance to correctly pick the better schedule when the PFS4 for two schedules are 50% and 65% (i.e., 15% difference in PFS4)

Table 14 shows the operating characteristics of the BOIN design for this trial with 4 scenarios defined by different DLT rates for 4 doses. These operating characteristics are based on 1,000 simulations of the trial using R package "BOIN", which is available from CRAN. The operating characteristics show that the design selects the true MTD with high probabilities and allocates more patients to the dose levels with the DLT rate closest to the target of 0.35.

Table 14 Operating Characteristics of the BOIN design

	Dose level				Number of pts	% early stopping
	-1	0	1	2		
True DLT rate	0.05	0.15	**0.35**	0.47		
Selection %	0.3	25.3	**54.3**	20.1	19.7	0.0
# pts treated	0.4	7.5	**8.5**	3.4		
True DLT rate	0.12	**0.35**	0.44	0.55		
Selection %	16.2	**59.3**	21.2	3.3	18.5	0.0
# pts treated	4.0	**9.7**	3.9	1.0		
True DLT rate	**0.35**	0.50	0.55	0.62		
Selection %	**57.0**	28.3	4.3	0.2	17.1	10.2
# pts treated	**8.2**	7.4	1.4	0.1		
True DLT rate	0.05	0.10	0.16	**0.35**		
Selection %	0.1	4.4	24.2	**71.3**	20.7	0.0
# pts treated	0.2	4.6	7.2	**8.7**		

In phase I, for each arm, the maximum sample size of 24 patients will be used for dose finding. As there are 4 doses, on average at least 6 patients will be treated at the MTD. After the MTD is selected, cohort expansion will be conducted at the MTD until the total number of patients treated at the MTD reaches 18 patients (including the patients who have been treated in the dose-finding part). Thus, no more than 12 additional patients will be enrolled during the cohort expansion phase. Therefore, the maximum number of patients for phase I, including dose finding and cohort expansion, is 24+12=36 patients per treatment arm, and 72 in total for two treatment arms.

During the interim analysis of the Phase I study, we identified TG02 of 250 mg as the MTD in both Arm 1 and Arm 2. Cohort expansion was conducted independently at each of the MTD into treatment arms, and the PSF4 was found to be 38% in Arm 1 and 25% in Arm 2. Therefore, based on the preplanned analysis, the DD TMZ dosing schedule is considered a “winner” to be used in Phase II study. However, a better PFS was found in patients who received TG02 at dose level 0, compared to those received TG02 at dose level 1, which is MTD, in Arm 1, the winner arm, suggesting the lower dose with the scheduled TMZ may be sufficient if not more beneficial for patients with recurrent high grade astrocytoma. However, we cannot make the conclusion based on the small sample number in Arm 1 at dose level 0. Therefore, we would like to continue enrolling to the maximum, allow the additional 12 patients enrolled to be treated with TG02 at dose level 0 combined with dd TMZ.

We do not plan to change the study design. The primary endpoint is still toxicity. The objective is to add a small cohort expansion to better characterize the dose-toxicity relationship and obtain some preliminary efficacy readout to determine RP2D. If PFS4 month difference between MTD and (MTD-1) is less than 5%, select the lower dose, I.e., MTD-1, as the RP2D; otherwise select the MTD as RP2D.

There should not be safety concerns because additional patients will be treated with TG02 at a dose that is lower than the MTD that was identified previously.

The purpose for enrolling more patients is to further define a dosage not only safe but optimal for the patients before we move to Phase II study. Only AE and PFS4 will be recorded in these subjects.

10.2 PHASE II COMPONENT:

A randomized phase II trial will be used to compare the combination of Zotiraciclib (TG02) plus TMZ (selected from phase I) with TMZ alone in patients with recurrent grade III or IV malignant glioma. The TMZ alone arm will use the same dosing schedule as it is in the combination arm. The primary endpoint is progression free survival. We will enroll at least 10 patients and at most 40 patients for each arm at a rate of 4 patients per month. Patients will be equally randomized into two arms. Patients' KPS, MGMT promoter methylation status of the tumors and the presence of measurable disease at study entry will be used as stratification factors. We will follow all patients for at least 12 months, and monitor futility and toxicity as described below. We will use the methods described by Thall et al., to monitor progression-free survival (PFS), and we will stop enrolling patients early if, based on the available data, we have reason to believe that the median PFS of the Zotiraciclib (TG02) plus TMZ arm is less than that of the TMZ arm.[50] Formally, we will stop enrolling patients early if

$$\Pr(\text{median PFS}_{\text{TG02+TMZ}} \geq \text{median PFS}_{\text{TMZ}} \mid \text{data from the trial}) < 0.78,$$

The futility monitoring will be conducted after enrolling 20, 40 and 60 patients (for two arms combined). Below are the operating characteristics of the above futility monitoring rule, assuming that the median PFS of the TMZ alone arm is 3 months.

Table 15 Operating Characteristics of the Futility Monitoring rule

	Median PFS for Zotiraciclib (TG02) +TMZ (months)				
	1	2	3	4	5
Early stop probability	0.999	0.812	0.374	0.170	0.097
Average sample size	23.7	42.1	62.6	70.9	74.7

Similar Bayesian methods will also be used to monitor the rate of DLT. We will monitor the two arms independently. Specifically, we will stop enrolling patients if the Pr(DLT > 35% | data) > 0.9. That is, we will stop enrolling patients if the data indicate that there is more than 90% chance that the true DLT rate is higher than 35%. This decision rule gives the following stopping rule, assuming a Beta (0.1, 0.4) prior distribution for DLT rate,

Stop enrolling pts if [# of pts with DLT] / [# of pts evaluated] ≥ 4/5, 6/10, 10/20, and 15/30.

Below are the operating characteristics of the toxicity monitoring rule.

Table 16: Operating Characteristics of the Toxicity Monitoring Rule

	True toxicity rate				
	0.2	0.3	0.4	0.5	0.6

Early stop probability	0.014	0.096	0.345	0.716	0.939
Average sample size	39.6	37.3	31.0	21.3	13.5

At progression, patients on the control arm will be offered treatment with Zotiraciclib (TG02) in combination with continued treatment with TMZ. If an effective salvage regimen, this may impact the secondary OS endpoint, but will not affect the primary endpoint of the study. The maximum number is 80 patients.

Statistical Analysis Plan

We will use descriptive statistics to summarize the demographic and clinical characteristics of patients. We will tabulate adverse events by grade by dose level and overall. For the cohort expansion and phase II component, we will estimate the proportion of patients with response with 95% exact binomial confidence intervals, and use descriptive statistics to summarize response duration. We will estimate PFS/OS with the Kaplan-Meier product limit estimator. Log-rank test will be used to compare the PFS/OS between Zotiraciclib (TG02)+TMZ and TMZ.

Changes of symptom burden (i.e., MDASI-BT) through study time points will be assessed by paired t-tests and linear mixed model. In the mixed model, subject-specific random intercept will be used to account for within-subject correlation, and measurement time will be included as a covariate. A sample size of 40 patients has 80% power to detect an effect size of 0.454 between time points using a two-tailed test with an alpha of 0.05. The two-sample t-test and linear mixed model will be used to evaluate differences in symptom burden using the MDASI-BT between Zotiraciclib (TG02) plus TMZ and TMZ. Cox proportional hazard model will be used to evaluate the relationship between MDASI-BT and disease progression. Logistic regression will be used to evaluate the relationship between MDASI-BT and tolerance to treatment.

Treatment effects will be evaluated by advanced MRI imaging study. Perfusion and diffusion – based MRI parameters, including but not restricted to blood volume, blood flow mean and median Apparent Diffusion Coefficient (ADC) value will be analysed. Results will be summarized. The mean and median value at different time points will be tabulated. Changes in such parameters will be analysed for statistical significance and correlate with the disease status.

10.2.1 Patient Reported Outcomes

Received MDASI-BT form will be checked versus the timing schedule and considered as valid if they fall within ten days of the scheduled assessment window. Compliance rates will be calculated as the number of received valid forms over the number of expected forms. Differences between groups in compliance will be tested by use of Fisher's exact test at every time point.

We will use descriptive statistics to describe how patients rate symptom severity and interference with function at each time point. Error bar graphs for each of the symptoms will be constructed at each time point. The proportion of patients rating their symptoms to be 5 or greater (on a 0-10 scale) will also be reported. We will construct individual patient profiles for each of the selected symptoms to describe the individual patients' patterns of change over time. We will calculate the mean symptom severity, and mean symptom interference at the time of clinical evaluation. Estimates of differences in the mean symptom severity and mean symptom interference between responders and non-responders will be estimated in the intent to treat population. All patients

with at least one valid questionnaire will be included in the analyses. Questionnaires completed at study registration will be considered baseline. All questionnaire data received after randomization will be used in the primary analyses.

Differences of at least 2 points will be classified as the minimum clinically meaningful change in the symptom severity and symptom interference measures. For example, an increase of 2 points or more would mean a moderate improvement, whereas a decrease of 2 points or more would be interpreted as moderate worsening. For individual symptoms, a rise in a symptom score means deterioration, whereas a reduced score means improvement of the specific symptom.

11 COLLABORATIVE AGREEMENTS

11.1 AGREEMENT TYPE

The study agent, Zotiraciclib (TG02) is provided by Adastra Pharmaceuticals, Inc. under a Clinical Trials Agreement (CTA #01007-16). Results or study data may be communicated to CTA partner, Adastra Pharmaceuticals, Inc. according to the terms of the NIH- Advanced Accelerator Applications SA CTA. There is an executed Material Transfer Agreement (MTA#40216-15) with Adastra Pharmaceuticals.

12 HUMAN SUBJECTS PROTECTIONS

12.1 RATIONALE FOR SUBJECT SELECTION

This study was designed to include women and minorities but was not designed to measure differences of intervention effects. Males and females will be recruited with no preference to gender. No exclusion to this study will be based on race. Minorities will actively be recruited to participate.

12.2 PARTICIPATION OF CHILDREN

Because no dosing or adverse event data are currently available on the use of TMZ in combination with Zotiraciclib (TG02) in subjects <18 years of age, children are excluded from this study.

12.3 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT

Adults unable to give consent are excluded from enrolling in the protocol. However, re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (a potential for reduction in their tumor volume, which may or may not have favorable impact on symptoms and/or survival), all subjects will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) for evaluation as needed for the following: an independent assessment of whether an individual has the capacity to provide consent; assistance in identifying and assessing an appropriate surrogate when indicated; and/or an assessment of the

capacity to appoint a surrogate. For those subjects that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in NIH HRPP SOP 14E for appointing a surrogate decision maker for adult subjects who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.

12.4 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

12.4.1 The primary risk to patients participating in this research study is from toxicity of the Zotiraciclib (TG02) and TMZ as described in the protocol.

12.4.2 Alternative Approaches or Treatments

Patients will be consented verbally and in writing regarding the risks and benefits of this trial, the treatment requirements, and alternative approaches to entering on this trial.

12.4.3 Procedure for Protecting Against or Minimizing any Potential Risks

All care will be taken to minimize side effects, but they can be unpredictable in nature and severity. This study may involve risks to patients, which are currently unforeseeable. All patients will have blood tests, examinations and scans as described in the study calendar (Section **3.8**). Patients will also be required to have a local physician to provide long-term care and to monitor for complications. If patients suffer any physical injury as a result of the participation in this study, immediate medical treatment is available at the Clinical Center, National Cancer Institute, Bethesda, Maryland. Although no compensation is available, any injury will be evaluated and treated in keeping with the benefits or care to which patients are entitled under applicable regulations.

12.4.4 Provisions for Monitoring Data Collection to Ensure Safety of Subjects

As information is gathered from this trial, clinical results will be shared with patients as they become available. Laboratory and clinical data will be frequently gathered and any new significant finding(s) found during the course of the research, which may affect a patient's willingness to participate further, will be explained. Confidentiality of information concerning participants will be maintained, including in all publications and presentations resulting from this study. Names of participants and/or material identifying participants will not be released without permission, except as such release is required by law. Records at the National Cancer Institute are maintained according to current legal requirements, and are made available for review, as required by the Food and Drug Administration or other authorized users, only under the guidelines established by the Federal Privacy Act.

12.5 RISKS/BENEFITS ANALYSIS

12.5.1 Benefits

The potential benefit to a patient on this study is a reduction in the bulk of their tumor and improvement in cancer lesions, which may or may not have favorable impact on symptoms and/or survival.

12.5.2 Risks

Risks include the possible occurrence of any of a range of side effects which are listed in the Consent Document or this protocol document. Frequent monitoring for adverse effects will help to minimize the risks associated with administration of the study agents.

12.5.3 MR Imaging with gadolinium

The MRIs to be done in this study will involve contrast. The risks associated with MRIs and contrast are discussed in the consent form.

Although MR imaging with gadolinium has been used as part of the standard care for patients with high grade gliomas, long term toxicities of gadolinium as a consequence of gadolinium accumulation in the body has been reported. All investigators will ascertain the MR imaging history of all prospective study participants to determine the extent of prior exposure to gadolinium contrast. Radiology will be consulted if there is a history of repeated gadolinium exposure.

12.5.4 Risks/Benefits Analysis

Given the potential benefits and the efforts to minimize risk with the administration of this combination, this protocol involves greater than minimal risk, but presents the potential for direct benefit to individual subjects, including those able to consent as well as those that may lose the capacity to do so over the course of the trial.

12.6 CONSENT AND ASSENT PROCESS AND DOCUMENTATION

The informed consent document will be provided to the participant for review prior to consenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomforts and benefits. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to entry onto the study.

12.6.1 The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved remote platforms) per discretion of the designated study investigator and with the agreement of the participant/consent designee(s). Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant/consent designee, when in person) will be located in a private area (e.g., clinic consult room). When consent is conducted remotely, the participant/consent designee will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed. Request for Waiver of Consent for Screening Activities

Prior to the subject signing the consent for this study pre-screening activities listed in section **2.2.1** may be performed.

We request a waiver of consent for these activities as they involve only minimal risk to the subjects. A waiver will not adversely affect the rights and welfare of the subjects given that the

activities are only intended to determine suitability for screening for participation in research protocols. These activities could not practicably be carried out without the wavier as central recruiting services, utilized in the NIH Clinical Center, perform pre-screening activities for multiple studies and obtaining consent for each one is beyond their resources. The subjects will be provided with additional pertinent information after participation as they will be informed whether or not they are eligible to sign a consent for additional screening.

13 PHARMACEUTICAL INFORMATION

13.1 TEMOZOLOMIDE (TEMODAR®; METHAZOLASTONE)

For more information, please refer to the Package Insert.

13.1.1 Source

Temozolomide will be purchased from commercial sources to the NIH CC Pharmacy.

13.1.2 Formulation and preparation

Temozolomide Capsules are available in 5-mg, 20-mg, 100-mg, 140-mg, 180-mg, and 250- mg strengths. The capsules contain a white capsule body with a color cap, and the colors vary based on the dosage strength.

Each strength of TEMODAR must be dispensed in a separate vial or in its original package (one strength per one container). Follow the instructions below: Based on the dose prescribed, determine the number of each strength of TEMODAR capsules that are needed. Label each container with the appropriate number of capsules to be taken each day. Dispense to the patient, making sure each container lists the strength (mg) per capsule and that he or she understands to take the appropriate number of capsules of TEMODAR from each package or vial to equal the total daily dose prescribed by the physician. TEMODAR is store at 25°C, excursions permitted to 15-30°C.

13.1.3 Contraindications

There are no dietary restrictions for patients taking TEMODAR. TEMODAR may affect testicular function, so male patients should exercise adequate birth control measures. TEMODAR may cause birth defects. Female patients should avoid becoming pregnant while receiving this drug. Women who are nursing prior to receiving TEMODAR should discontinue nursing. It is not known whether TEMODAR is excreted into breast milk. Because many drugs are excreted in human milk, and because of the potential for serious adverse reactions in nursing infants and tumorigenicity shown for temozolomide in animal studies, a decision should be made whether to discontinue nursing or to discontinue the drug, taking into account the importance of TEMODAR to the mother.

13.1.4 Administration procedures

Patients should take each day's dose with a full glass of water at the same time each day. Taking the medication on an empty stomach or at bedtime may help ease nausea. If patients are also taking anti-nausea or other medications to relieve the side effects associated with TEMODAR,

they should be advised to take these medications 30 minutes before they take TEMODAR. Temozolomide causes the rapid appearance of malignant tumors in rats. Patients SHOULD NOT open or split the capsules. If capsules are accidentally opened or damaged, rigorous precautions should be taken with the capsule contents to avoid inhalation or contact with the skin or mucous membranes. The medication should be kept away from children and pets. The TEMODAR capsules should be swallowed whole and NEVER CHEWED.

13.1.5 Side Effects

The most common adverse reactions (≥10% incidence) are: alopecia, fatigue, nausea, vomiting, headache, constipation, anorexia, convulsions, rash, hemiparesis, diarrhea, asthenia, fever, dizziness, coordination abnormal, viral infection, amnesia, and insomnia. The most common Grade 3 to 4 hematologic laboratory abnormalities (≥10% incidence) that have developed during treatment with temozolomide are: lymphopenia, thrombocytopenia, neutropenia, and leukopenia.

The adverse reaction from clinical trials that are conducted in different patient population are summarized as following:

Newly Diagnosed Glioblastoma: During the concomitant phase (TEMODAR+radiotherapy), adverse reactions including thrombocytopenia, nausea, vomiting, anorexia, and constipation were more frequent in the TEMODAR+RT arm. The incidence of other adverse reactions was comparable in the two arms. The most common adverse reactions across the cumulative TEMODAR experience were alopecia, nausea, vomiting, anorexia, headache, and constipation. Forty-nine percent (49%) of patients treated with TEMODAR reported one or more severe or life-threatening reactions, most commonly fatigue (13%), convulsions (6%), headache (5%), and thrombocytopenia (5%). Overall, the pattern of reactions during the maintenance phase was consistent with the known safety profile of TEMODAR. Myelosuppression (neutropenia and thrombocytopenia), which is a known dose-limiting toxicity for most cytotoxic agents, including TEMODAR, was observed. When laboratory abnormalities and adverse reactions were combined, Grade 3 or Grade 4 neutrophil abnormalities including neutropenic reactions were observed in 8% of the patients, and Grade 3 or Grade 4 platelet abnormalities, including thrombocytopenic reactions, were observed in 14% of the patients treated with TEMODAR.

Refractory Anaplastic Astrocytoma: The incidence of adverse reactions in the 158 patients in the anaplastic astrocytoma study for whom data are available. In the absence of a control group, it is not clear in many cases whether these reactions should be attributed to temozolomide or the patients' underlying conditions, but nausea, vomiting, fatigue, and hematologic effects appear to be clearly drug-related. The most frequently occurring adverse reactions were nausea, vomiting, headache, and fatigue. The adverse reactions were usually NCI Common Toxicity Criteria (CTC) Grade 1 or 2 (mild to moderate in severity) and were self-limiting, with nausea and vomiting readily controlled with anti-emetics. The incidence of severe nausea and vomiting (CTC Grade 3 or 4) was 10% and 6%, respectively. Myelosuppression (thrombocytopenia and neutropenia) was the dose-limiting adverse reaction. It usually occurred within the first few cycles of therapy and was not cumulative. Myelosuppression occurred late in the treatment cycle and returned to

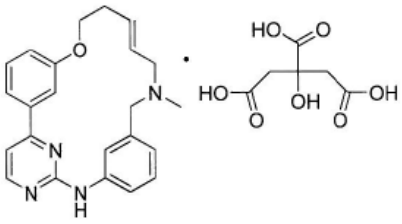
normal, on average, within 14 days of nadir counts. The median nadirs occurred at 26 days for platelets (range: 21-40 days) and 28 days for neutrophils (range: 1-44 days). Only 14% (22/158) of patients had a neutrophil nadir and 20% (32/158) of patients had a platelet nadir, which may have delayed the start of the next cycle. Less than 10% of patients required hospitalization, blood transfusion, or discontinuation of therapy due to myelosuppression.
In clinical trial experience with 110 to 111 women and 169 to 174 men (depending on measurements), there were higher rates of Grade 4 neutropenia (ANC less than 500 cells/μL) and thrombocytopenia (less than 20,000 cells/μL) in women than men in the first cycle of therapy (12% vs. 5% and 9% vs. 3%, respectively). In the entire safety database for which hematologic data exist (N=932), 7% (4/61) and 9.5% (6/63) of patients over age 70 experienced Grade 4 neutropenia or thrombocytopenia in the first cycle, respectively. For patients less than or equal to age 70, 7% (62/871) and 5.5% (48/879) experienced Grade 4 neutropenia or thrombocytopenia in the first cycle, respectively. Pancytopenia, leukopenia, and anemia have also been reported.

13.2 ZOTIRACICLIB (TG02)

13.2.1 Description of Zotiraciclib (TG02) Citrate

The chemical name for Zotiraciclib (TG02) citrate is (16E)-14-Methyl-20-oxa-5,7,14,26-tetraaza-tetracyclol[19.3.1.1(2,6).1(8,12)]heptacosa-1(25),2(26),3,5,8(27),9,11,16,21,23-decaene − citric acid. The molecular formula of Zotiraciclib (TG02) citrate is $C_{23}H_{24}N_4O \bullet C_6H_8O_7$ and it has a molecular weight of 564.58 (TG02 citrate salt) and 372.46 (TG02 base).

The structure is as follows:



	TG02 Citrate
Trade name	Zotiraciclib citrate
Manufacturer	Adastra Pharmaceuticals, Inc.
Strength (mg)	50, 150
Dosing instructions	Take appropriate number of capsules with 8oz. water while fasting (i.e., at least 1 hour before or 2 hours after a meal). Capsules should not be crushed or broken for administration.
Route	Oral
Dosage form	Capsule
Storage	Store at 25°C (77°F); excursions permitted 15°C to 30°C (59°F to 86°F)

13.2.2 Source

Zotiraciclib (TG02) is an investigational agent. The IND holder is the NCI Center for Cancer Research. Adastra Pharmaceutical, Inc. will provide Zotiraciclib (TG02) citrate capsules for this protocol.

13.2.3 Toxicity

All toxicities observed in patients are from the data provided in the Investigators Brochure Version 6. All data are derived from clinical studies that are summarized in the table 6 in Section 1.

11.2.3.1 Single agent Zotiraciclib (TG02) in Acute Leukemia Patients

Fifty-five acute leukemia patients were evaluated for the safety and efficacy of single agent Zotiraciclib (TG02). 96% (53/55) patients experienced Treatment Emergent Adverse Events (TEAE). Most frequently reported TEAEs (43/55 patients 78%) occurred in the System Organ Class of Gastrointestinal Disorders. They were also frequently reported in the following System Oran Classes: General Disorders and Administration Site Conditions (23/55 patients, 42%), Metabolism and Nutrition Disorders (22/55 patients, 40%), Nervous system Disorders (17/55patients, 31%), Respiratory, Thoracic and Mediastinal Disorders (14/55 patients, 25%), and Skin and Subcutaneous Tissue Disorders (12/55 patients, 22%).

The most frequently reported TEAEs were as follows: nausea (32/55 patients, 58%), vomiting (21/55 patients 38%), fatigue (15/55 patients, 27%) and diarrhea, decreased appetite and abdominal pain (13/55 patients each, 24%).

Thirty-nine patients (39/55 patients, 71%) had ≥1 treatment-related TEAEs. Five patients (9%) experienced grade 5/fatal TEAE and all five were assed as unrelated to the study drug by the investigator. Three patients (5%) had grade 4/life threatening TEAE, including hyperglycemia, cardio-respiratory arrest and enterococcal sepsis. All events were assed as unrelated to the study drug. The most commonly reported grade 3TEAEs were fatigue (11%) and nausea (7%).

When TEAEs were analyzed by their maximum severity, regardless of causality, five acute leukemia patients (5/55 [9%]) who were administered single agent Zotiraciclib (TG02) experienced a Grade 5/fatal TEAE (Schedule A, 3/27 patients [11%]; Schedule B, 2/22 [9%]; Schedule D, 0/6 [0%]). The Preferred Terms for the Grade 5 TEAEs were pneumonia (2 patients), sepsis, intracranial hemorrhage, and cardio-respiratory arrest. All five events were assessed as unrelated to study drug by the investigator.
Three acute leukemia patients (3/55 [5%]) administered single agent Zotiraciclib (TG02) experienced a Grade 4/life-threatening TEAE (Schedule A, 1/27 patients [4%]; Schedule B, 2/22 patients [9%]; Schedule D, 0/6 patients [0%]). The Preferred Terms for the Grade 4 TEAEs were hyperglycemia, cardio-respiratory arrest and enterococcal sepsis. All events were assessed as unrelated to study drug by the investigator. The most frequently reported Grade 3 TEAEs were fatigue (6/55 patients [11%]) and nausea (4/55 patients [7%]) occurring on Schedules A and B. No Grade 3 or higher fatigue, nausea, vomiting or diarrhea occurred on Schedule D.

Serious Adverse Events: Fifteen acute leukemia patients (15/55 [27%]) administered single agent Zotiraciclib (TG02) experienced ⩾1 SAE (Schedule A, 5/27 patients [19%]; Schedule B, 8/22 patients [36%]; Schedule D 2/6 patients [33%]). No individual body system had an incidence of

SAEs of ⩾10%. No significant differences were noted between schedules. Four patients (4/55 patients [7%]) experienced ⩾ 1 serious suspected adverse reaction considered related to study drug by the investigator (Schedule A, 1/27 patients [4%]; Schedule B, 3/22 patients [14%]; Schedule D, (0/6 patients [0%]).

- Patient 03-002 (Schedule A, 10 mg) experienced two occurrences of related nausea resulting in hospitalization and three occurrences of related vomiting resulting in hospitalization (2 of the occurrences occurred concomitantly with the related nausea). Study drug was interrupted. The patient recovered without sequelae from all events.
- Patient 03-018 (Schedule B, 50 mg) experienced related vomiting that resulted in hospitalization. Study drug was interrupted due to this event.
- Patient 01-053 (Schedule B, 150 mg) experienced two occurrences of related fatigue which required hospitalization. The first occurrence of fatigue occurred during Cycle 1 and was considered a DLT. Study drug was withheld and resumed at the start of Cycle 2 at 100 mg. The second occurrence of fatigue was in Cycle 2. Study drug was held and resumed at the start of Cycle 3 at 100 mg on Days 1-3 and 8-10, a reduced schedule. The patient came off study at the end of Cycle 4 for progressive disease. This patient also experienced the non-related SAE of respiratory syncytial virus infection. The patient recovered without sequelae from all events.
- Patient 03-057 (Schedule B, 150 mg) experienced the related SAE of severe failure to thrive, which required hospitalization. Study drug was interrupted and subsequently discontinued the study due to this event. The patient recovered without sequelae from the SAE.

Study Drug Discontinuations Due to TEAEs: Seven acute leukemia patients (7/55 [13%]) administered single agent Zotiraciclib (TG02) discontinued study drug due to a TEAE (Schedule A, 3/27 [11%]; Schedule B, 3/22 patients [14%]; Schedule D 1/6 patients [17%]). No individual body system had an incidence of SAEs of ⩾10%. No significant differences were noted between schedules. The Preferred Terms for these TEAEs were vomiting, pneumonia, maculopapular rash, dyspepsia, nausea, fatigue and balance disorder (in one patient) and stomatitis.

Deaths: Five patients (5/55 [9%]) experienced a Grade 5/ fatal TEAE (Schedule A, 3/27 patients [11.1%]; Schedule B, 2/22 patients [4.5%]; Schedule D, (0/6 patients [0%]).

- Patient 01-026 (Schedule A, 50 mg Zotiraciclib (TG02)) died of pneumonia. The patient had discontinued study drug and the event occurred during the follow-up period. The event was assessed as not related to study drug by the investigator.
- Patient 01-044 (Schedule A, 70 mg Zotiraciclib (TG02)) died of pneumonia. The event was assessed as not related to study drug by the investigator.
- Patient 03-015 (Schedule A, 30 mg Zotiraciclib (TG02)) died due to sepsis and refractory AML. The patient had discontinued study drug and the event occurred during the follow-up period. The event was assessed as not related to study drug.
- Patient 01-059 (Schedule B, 150 mg Zotiraciclib (TG02)) died due to intracranial hemorrhage. The patient had discontinued study drug and the event occurred during the follow-up period. The event was assessed as not related to study drug by the investigator.

- Patient 03-049 (Schedule B, 100 mg Zotiraciclib (TG02) died due to cardiopulmonary arrest. The event was assessed as not related to study drug by the investigator.

11.2.3.2 Single agent Zotiraciclib (TG02) in Multiple Myeloma (MM) Patients

Eighteen multiple myeloma patients administered single agent Zotiraciclib (TG02) were evaluated for safety. All patients had discontinued from the study as of the data cut-off date. The reasons for discontinuation were withdrawal of consent (7/18 patients [39%]), progressive disease (7/18 patients [39%]), other (2/18 patients [11%]), adverse event (1/18 patients [6%]) and investigator decision (1/18 patients [6%]). Most frequently reported TEAEs (17/18 patients 94%) occurred in the System Organ Class of Gastrointestinal Disorders. The most frequently reported TEAEs were as follows: diarrhea (10/18 patients, 56%), nausea and fatigue (9/18 patients, 50%), vomiting and constipation (7/18 patients each, 39%), hypophosphatemia (6/18 patients, 33%), decreased appetite and hypokalemia (5/18 patients each, 28%). The incidence of the TEAEs constipation, hypophosphatemia, decreased appetite and hypokalemia did not differ significantly between the dosing schedules. Diarrhea, fatigue, nausea and vomiting occurred more frequently on Schedule G than Schedule C. Higher doses were administered in Schedule G (100 and 150 mg) than in Schedule C (50 and 70 mg). Fatigue and vomiting occurred more frequently on Schedule G than Schedule H. A less dose-intense schedule was administered in Schedule H (BIW for 3 weeks) than in Schedule G (TIW for 3 weeks) although the number of patients in Schedule H is small.

• Diarrhea: Schedule C, 2 pts [33%]; Schedule G, 6 pts [78%]; Schedule H, 2 pts [67%]
• Fatigue: Schedule C, 1 pts [17%]; Schedule G, 7 pts [77%]; Schedule H, 1 pt [33%]
• Nausea: Schedule C, 1 pts [17%]; Schedule G, 6 pts [67%]; Schedule H, 2 pts [67%]
• Vomiting: Schedule C, 0 pts [0%]; Schedule G, 6 pts [67%]; Schedule H, 1 pt [33%]

Fifteen patients (15/18 patients, 83%) had ≥1 treatment-related TEAEs. One patients (17%) experienced grade 5/fatal TEAE and the event was assed as unrelated to the study drug by the investigator. Five patients (28%) had grade 4/life threatening TEAE, including GI hemorrhage, thrombocytopenia, neutrophil count decreased, platelet count reduced and hyperuricemia. All events were assed as unrelated to the study drug. The most commonly reported grade 3TEAEs were hypophosphatemia (28%).

Serious Adverse Events: Five multiple myeloma patients (5/18 patients [28%]) administered single agent Zotiraciclib (TG02) experienced ⩾1 SAE (Schedule C, 4/6 patients [67%]; Schedule G, 0/9 patients [0%]; Schedule H, 1/3 patients [33%]). Each of the SAEs (GI hemorrhage and respiratory failure [in the same patient], fatigue, pneumonia, bone pain and pneumonia streptococcal) were experienced by 1 patient each. One patient (1/18 [6%]) experienced a serious suspected adverse reaction considered related to study drug by the investigator. Patient 02-058 (Schedule C, 70 mg) experienced Grade 3 related fatigue resulting in hospitalization. This event was considered a DLT. Study drug was interrupted and then discontinued due to this event. The patient recovered without sequelae from the event.

Study Drug Discontinuations Due to TEAEs: Two multiple myeloma patients (2/18 [11%]) administered single agent Zotiraciclib (TG02) discontinued study drug due to a TEAE (Schedule C, 2/6 patients [33%], Schedules G and H (0/12 patients [0%]). One patient discontinued study drug due to fatigue that was assessed by the investigator as related to study drug and a DLT. The

patient recovered without sequelae from the event. The second patient discontinued study drug due to musculoskeletal bone pain that was assessed by the investigator as not related to study drug. The patient recovered with sequela from the event.

Deaths: One multiple myeloma patient (1/18 [16.7%]) administered single agent Zotiraciclib (TG02) experienced Grade 5 fatal respiratory failure. Patient 05-045 (Schedule C, 50 mg) died of respiratory failure. The event was assessed as not related to study drug by the investigator and related to the patient's underlying disease state (i.e., progressive disease).

11.2.3.3 Single Agent Zotiraciclib (TG02) in Chronic Lymphocytic Leukemia (CLL) patients

Only eight CLL patients administered single agent Zotiraciclib (TG02) were evaluated for the drug safety. Seven of them (88%) experienced ⩾1 TEAEs.

Most frequently reported TEAEs (17/18 patients 94%) occurred in the System Organ Class of Gastrointestinal Disorders. The most frequently reported TEAEs were as follows: nausea (6/8 patients, 75%), diarrhea (4/8 patients, 50%), vomiting, constipation, cough and dyspnea (3/8 patients each, 38%). Four patients developed treatment –related TEAEs with nausea being the most frequently reported treatment –related TEAEs. No dose-related trends were noted due to the small number of patients.

No CLL patients experienced a Grade 5/ fatal TEAE. Three CLL patients (3/8 patients [38%]) administered single agent Zotiraciclib (TG02) experienced Grade 4/ Life-Threatening TEAEs. Preferred Terms for the Grade 4 TEAEs were sepsis and thrombocytopenia (1 patient), sepsis, pneumonia staphylococcal and staphylococcal bacteremia (1 patient), thrombocytopenia, neutropenia and hypotension. The events of sepsis and thrombocytopenia occurring in the same patient were assessed as related to study drug by the investigator. The other Grade 4 TEAEs were assessed as unrelated to study drug by the investigator. No Grade 3 TEAE was experienced by more than one patient (1/8 [13%]).

Serious Adverse Events: Three CLL patients (3/8 [38%]) administered single agent Zotiraciclib (TG02) experienced ⩾ 1 SAE. The body systems in which the SAEs occurred were Infections and Infestations (2/8 patients [25%]), Blood and Lymphatic System Disorders (1/8 patients [13%]) and Psychiatric Disorders (1/8 patients [13%]). Only the SAE of sepsis was experienced by more than one patient (2/8 patients [25%]). Two CLL patients (2/8 [25%]) experienced a serious suspected adverse reaction considered by the investigator to be related to study drug.

- Patient 200-005 (Schedule A, 70 mg) experienced related confusion state resulting in hospitalization. This event was Grade 2 and did not meet the criteria for a DLT. Study drug was interrupted. The patient recovered without sequelae from the event.
- Patient 400-010 (Schedule A, 100 mg) experienced related sepsis, thrombocytopenia and febrile neutropenia. Sepsis was considered a DLT. Study drug was interrupted and restarted at 70 mg, a reduced dose.

Study Drug Discontinuations Due to TEAEs: One CLL patient (1/8 [13%]) administered single agent Zotiraciclib (TG02) discontinued study drug due to a TEAE. Patient 100-003 discontinued study drug due to pneumonia staphylococcal, staphylococcal abscess and staphylococcal bacteremia. The patient recovered without sequelae from the events.

Deaths: No CLL subjects administered single agent Zotiraciclib (TG02) died on study.

13.2.4 Formulation and preparation

Zotiraciclib (TG02) citrate capsules will be provided as formulated dry powder blend containing Zotiraciclib (TG02) citrate in hard gelatin capsules. Capsule strengths are expressed as Zotiraciclib (TG02). The formulation compositions and brief descriptions of the capsule products are shown in the following table. The capsule strengths (50 and 150mg) are distinguished by size and color, and the actual dose of Zotiraciclib (TG02) has been corrected for citrate salt (e.g., 50mg Zotiraciclib (TG02) is 76mg Zotiraciclib (TG02) citrate).

Table 17: Description of Zotiraciclib (TG02) Citrate Capsules

	Strength (mg)	
	50mg (TG02-50)	**150mg (TG02-150)**
Capsule size	2	0
Capsule color	Swedish orange	Light blue
Ingredients (mg/capsule)		
TG02 citrate	76.0	228.0
Silicified microcrystalline cellulose, NF	109.9	88.2
Hypromellose 2910, USP	10.5	18.0
Crospovidone, NF	10.5	18.0
Magnesium stearate, NF	1.1	1.8

13.2.5 Stability and Storage

Zotiraciclib (TG02) citrate capsules should be stored at room temperature 25°C (77°F), with excursions permitted at 15°C to 30°C (59°F to 86°F).

13.2.6 Administration procedures

Patients are instructed to swallow the appropriate number of capsules whole with 8oz. of water while fasting (i.e., at least 1 hour before or 2 hours after a meal). Capsules should not be crushed or broken for administration.

13.2.7 Incompatibilities

Please refer to section **13.1**. A copy of the drug brochure will be provided to the Pharmacy Department.

14 REFERENCES

1. Ostrom, Q.T., et al., *CBTRUS Statistical Report: Primary Brain and Central Nervous System Tumors Diagnosed in the United States in 2008-2012.* Neuro Oncol, 2015. **17 Suppl 4**: p. iv1-iv62.
2. Stupp, R., et al., *Radiotherapy plus concomitant and adjuvant temozolomide for glioblastoma.* N Engl J Med, 2005. **352**(10): p. 987-96.
3. Wong, E.T., et al., *Outcomes and prognostic factors in recurrent glioma patients enrolled onto phase II clinical trials.* J Clin Oncol, 1999. **17**(8): p. 2572-8.
4. Yung, W.K., et al., *Multicenter phase II trial of temozolomide in patients with anaplastic astrocytoma or anaplastic oligoastrocytoma at first relapse. Temodal Brain Tumor Group.* J Clin Oncol, 1999. **17**(9): p. 2762-71.
5. Louis, D.N., *Molecular pathology of malignant gliomas.* Annu Rev Pathol, 2006. **1**: p. 97-117.
6. Li, J., et al., *PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer.* Science, 1997. **275**(5308): p. 1943-7.
7. Fujisawa, H., et al., *Acquisition of the glioblastoma phenotype during astrocytoma progression is associated with loss of heterozygosity on 10q25-qter.* Am J Pathol, 1999. **155**(2): p. 387-94.
8. Goh, K.C., et al., *TG02, a novel oral multi-kinase inhibitor of CDKs, JAK2 and FLT3 with potent anti-leukemic properties.* Leukemia, 2012. **26**(2): p. 236-43.
9. Loyer, P., et al., *Role of CDK/cyclin complexes in transcription and RNA splicing.* Cell Signal, 2005. **17**(9): p. 1033-51.
10. Romano, G. and A. Giordano, *Role of the cyclin-dependent kinase 9-related pathway in mammalian gene expression and human diseases.* Cell Cycle, 2008. **7**(23): p. 3664-8.
11. Atkinson, J.M., et al., *An integrated in vitro and in vivo high-throughput screen identifies treatment leads for ependymoma.* Cancer Cell, 2011. **20**(3): p. 384-99.
12. Nechaev, S. and K. Adelman, *Pol II waiting in the starting gates: Regulating the transition from transcription initiation into productive elongation.* Biochim Biophys Acta, 2011. **1809**(1): p. 34-45.
13. Lam, L.T., et al., *Genomic-scale measurement of mRNA turnover and the mechanisms of action of the anti-cancer drug flavopiridol.* Genome Biol, 2001. **2**(10): p. RESEARCH0041.
14. Carter, B.Z., et al., *Small-molecule XIAP inhibitors derepress downstream effector caspases and induce apoptosis of acute myeloid leukemia cells.* Blood, 2005. **105**(10): p. 4043-50.
15. Quinn, B.A., et al., *Targeting Mcl-1 for the therapy of cancer.* Expert Opin Investig Drugs, 2011. **20**(10): p. 1397-411.

16. Day, B.W., et al., *ELK4 neutralization sensitizes glioblastoma to apoptosis through downregulation of the anti-apoptotic protein Mcl-1.* Neuro Oncol, 2011. **13**(11): p. 1202-12.

17. Wagenknecht, B., et al., *Expression and biological activity of X-linked inhibitor of apoptosis (XIAP) in human malignant glioma.* Cell Death Differ, 1999. **6**(4): p. 370-6.

18. Rahl, P.B., et al., *c-Myc regulates transcriptional pause release.* Cell, 2010. **141**(3): p. 432-45.

19. Barboric, M., et al., *NF-kappaB binds P-TEFb to stimulate transcriptional elongation by RNA polymerase II.* Mol Cell, 2001. **8**(2): p. 327-37.

20. Bhat, K.P., et al., *Mesenchymal differentiation mediated by NF-kappaB promotes radiation resistance in glioblastoma.* Cancer Cell, 2013. **24**(3): p. 331-46.

21. Herms, J.W., et al., *c-myc oncogene family expression in glioblastoma and survival.* Surg Neurol, 1999. **51**(5): p. 536-42.

22. Malumbres, M. and M. Barbacid, *Cell cycle, CDKs and cancer: a changing paradigm.* Nat Rev Cancer, 2009. **9**(3): p. 153-66.

23. Simone, C. and A. Giordano, *New insight in cdk9 function: from Tat to MyoD.* Front Biosci, 2001. **6**: p. D1073-82.

24. Huang, C.H., et al., *CDK9-mediated transcription elongation is required for MYC addiction in hepatocellular carcinoma.* Genes Dev, 2014. **28**(16): p. 1800-14.

25. Alvarez-Fernandez, S., et al., *Potent antimyeloma activity of a novel ERK5/CDK inhibitor.* Clin Cancer Res, 2013. **19**(10): p. 2677-87.

26. Gunther, W., et al., *Temozolomide induces apoptosis and senescence in glioma cells cultured as multicellular spheroids.* Br J Cancer, 2003. **88**(3): p. 463-9.

27. Gratas, C., et al., *Bak and Mcl-1 are essential for Temozolomide induced cell death in human glioma.* Oncotarget, 2014. **5**(9): p. 2428-35.

28. Shiraishi, S., et al., *Influence of p53 mutations on prognosis of patients with glioblastoma.* Cancer, 2002. **95**(2): p. 249-57.

29. Oren, M. and V. Rotter, *Mutant p53 gain-of-function in cancer.* Cold Spring Harb Perspect Biol, 2010. **2**(2): p. a001107.

30. Trent, J., et al., *Evidence for rearrangement, amplification, and expression of c-myc in a human glioblastoma.* Proc Natl Acad Sci U S A, 1986. **83**(2): p. 470-3.

31. Cancer Genome Atlas Research, N., *Comprehensive genomic characterization defines human glioblastoma genes and core pathways.* Nature, 2008. **455**(7216): p. 1061-8.

32. Tohma, Y., et al., *PTEN (MMAC1) mutations are frequent in primary glioblastomas (de novo) but not in secondary glioblastomas.* J Neuropathol Exp Neurol, 1998. **57**(7): p. 684-9.

33. Austin, M. and S.J. Cook, *Increased expression of Mcl-1 is required for protection against serum starvation in phosphatase and tensin homologue on chromosome 10 null*

mouse embryonic fibroblasts, but repression of Bim is favored in human glioblastomas. J Biol Chem, 2005. **280**(39): p. 33280-8.

34. Radu, A., et al., *PTEN induces cell cycle arrest by decreasing the level and nuclear localization of cyclin D1.* Mol Cell Biol, 2003. **23**(17): p. 6139-49.
35. Siegel, D.S., et al., *A phase 2 study of single-agent carfilzomib (PX-171-003-A1) in patients with relapsed and refractory multiple myeloma.* Blood, 2012. **120**(14): p. 2817-25.
36. Bower, M., et al., *Multicentre CRC phase II trial of temozolomide in recurrent or progressive high-grade glioma.* Cancer Chemother Pharmacol, 1997. **40**(6): p. 484-8.
37. Bocangel, D.B., et al., *Multifaceted resistance of gliomas to temozolomide.* Clin Cancer Res, 2002. **8**(8): p. 2725-34.
38. Hirose, Y., M.S. Berger, and R.O. Pieper, *p53 effects both the duration of G2/M arrest and the fate of temozolomide-treated human glioblastoma cells.* Cancer Res, 2001. **61**(5): p. 1957-63.
39. Tolcher, A.W., et al., *Marked inactivation of O6-alkylguanine-DNA alkyltransferase activity with protracted temozolomide schedules.* Br J Cancer, 2003. **88**(7): p. 1004-11.
40. Wick, A., et al., *Efficacy and tolerability of temozolomide in an alternating weekly regimen in patients with recurrent glioma.* J Clin Oncol, 2007. **25**(22): p. 3357-61.
41. Han, S.J., et al., *Phase II trial of 7 days on/7 days off temozolmide for recurrent high-grade glioma.* Neuro Oncol, 2014. **16**(9): p. 1255-62.
42. Perry, J.R., et al., *Phase II trial of continuous dose-intense temozolomide in recurrent malignant glioma: RESCUE study.* J Clin Oncol, 2010. **28**(12): p. 2051-7.
43. Armstrong, T.S., et al., *Reliability and validity of the M. D. Anderson Symptom Inventory-Spine Tumor Module.* J Neurosurg Spine, 2010. **12**(4): p. 421-30.
44. Armstrong, T.S., et al., *Validation of the M.D. Anderson Symptom Inventory Brain Tumor Module (MDASI-BT).* J Neurooncol, 2006. **80**(1): p. 27-35.
45. Liu, S.a.Y., Ying, *Bayesian optimal interval designs for phase I clinical trials.* Journal of the Royal Statistical Society: Series C (Applied Statistics), 2015. **64**(3).
46. Liu, S. and Y. Yuan, *Bayesian optimal interval designs for phase I clinical trials.* Journal of the Royal Statistical Society: Series C (Applied Statistics), 2015. **64**(3): p. 507-523.
47. Wen, P.Y. and P.W. Marks, *Medical management of patients with brain tumors.* Curr Opin Oncol, 2002. **14**(3): p. 299-307.
48. Semrad, T.J., et al., *Epidemiology of venous thromboembolism in 9489 patients with malignant glioma.* J Neurosurg, 2007. **106**(4): p. 601-8.
49. Wen, P.Y., et al., *Updated response assessment criteria for high-grade gliomas: response assessment in neuro-oncology working group.* J Clin Oncol, 2010. **28**(11): p. 1963-72.
50. Thall, P.F., L.H. Wooten, and N.M. Tannir, *Monitoring event times in early phase clinical trials: some practical issues.* Clin Trials, 2005. **2**(6): p. 467-78.

15 APPENDICES

15.1 APPENDIX: PERFORMANCE STATUS CRITERIA

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.

15.2 APPENDIX: PREGNANCY & CONTRACEPTION

Pregnancy tests for females of childbearing potential and adequate methods of contraception

A female of childbearing potential (FCBP) is a sexually mature female who: 1) has not undergone a hysterectomy or bilateral oophorectomy; or 2) has not been naturally postmenopausal for at least 24 consecutive months (i.e., has had menses at any time in the preceding 24 consecutive months).

Pregnancy tests must occur within 14 days prior to initiation of study. FCBP must have a pregnancy test before each new cycle; at discontinuation of Zotiraciclib (TG02) and at Day 28 post the last dose of Zotiraciclib (TG02).

Required Pregnancy test: Serum β-HCG

Adequate method of contraception: one highly effective method or more than one of the additional methods.

Highly effective methods:

- Intrauterine device (IUD)
- Hormonal (birth control pills, injections, implants)
- Tubal ligation
- Partner's vasectomy
- Abstinence

Additional effective methods:

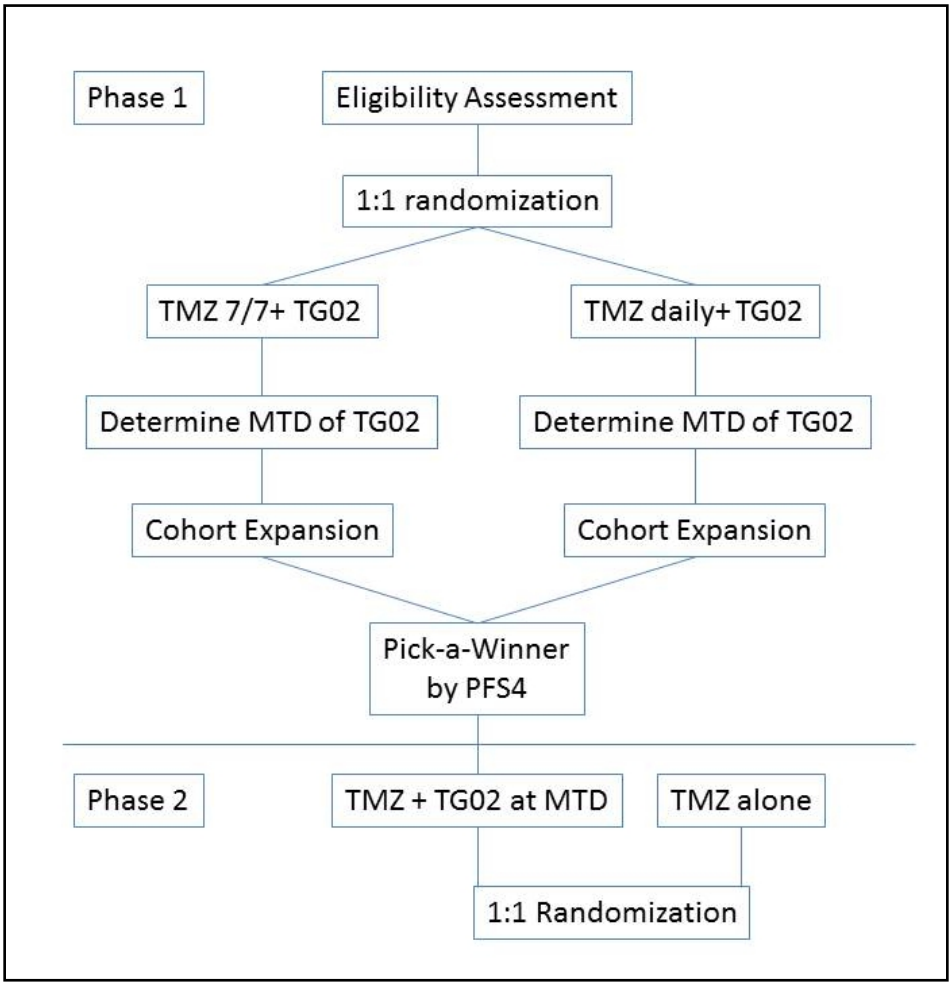
- Latex condom
- Diaphragm
- Cervical Cap

15.3 APPENDIX: CYP1A2 AND CYP3A4

Lists including medications and substances known or with the potential to interact with the CYP1A2 and CYP3A4 isoenzymes

- Known inhibitors of CYP1A2:
  - Acyclovir
  - fluvoxamine
- Known inhibitors of CYP3A4:
  - Atazanavir
  - Clarithromycin
  - indinavir
  - itraconazole
  - ketoconazole
  - nefazodone
  - nelfinavir
  - ritonavir
  - saquinavir
  - telithromycin
- Known inducers of CYP1A2:
  - omeprazole
  - insulin
  - cigarette smoking,
- Known inducers of CYP3A4:
  - rifampin and
  - carbamazepine
- Sensitive CYP1A2 substrates with narrow therapeutic indices:
  - theophylline and
  - tizanidine,
- Sensitive CYP2D6 substrates with narrow therapeutic indices:
  - thioridazine
  - tamoxifen

15.4 APPENDIX: STUDY FLOW CHART



15.5 APPENDIX: RANO

Criteria for Response Assessment Incorporating MRI and Clinical Factors

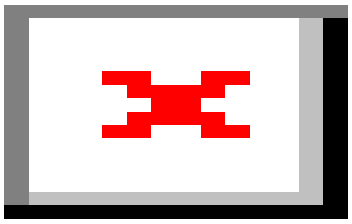
	CR	PR	SD	PD
T1-Gd+	None	≥50%	<50%↓- <25%↑	≥25%↑*
T2/FLAIR	Stable or ↓	Stable or ↓	Stable or ↓	↑*
New Lesion	None	None	None	Present*
Corticosteroids	None	Stable or ↓	Stable or ↓	NA
Clinical Status	Stable or ↑	Stable or ↑	Stable or ↑	↓*
Requirement for Response	All	All	All	Any*

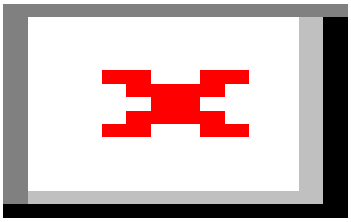
CR = Complete Response, PR = Partial Response, SD = Stable Disease, PD = Progressive Disease

* Progression occurs when any of these criteria are met present.

NA: An increase in steroid dose alone will not cause a determination of progression in the absence of clinical deterioration or radiographically documented lesion growth.

15.6 APPENDIX: DOSING SCHEDULE FOR BOTH TREATMENTS ARMS FOR PHASE I STUDY





15.7 APPENDIX: PILL DIARY

Dose-dense arm

Cycle 1

Patient Initials: _____Patient ID#: _____Phase___Cycle # 1:_____

This diary is for you to record that you took the drugs as instructed by your doctor. Please put a check mark or your initials after each dose. Please sign this diary at the end of the cycle. Bring the diary and all study drug bottles back to your next clinic visit.

TG02 AM **Zotiraciclib** **Frequency:** Only on Day 1, Day 12, Day 15, Day 26 *Cycle 1 only: extra dose on D -3					**Temozolomide** PM **Frequency**: 7days ON, 7 days OFF				
DAY #	**DATE**	**TIME**	**DOSE**	**INITIALS**	**DAY #**	**DATE**	**TIME**	**DOSE**	**INITIALS**
-3 *									
	off day								
	off day								
1					**1**				
	off day				**2**				
	off day				**3**				
	off day				**4**				
	off day				**5**				
	off day				**6**				
	off day				**7**				
	off day				8	off day			
	off day				9	off day			
	off day				10	off day			
	off day				11	off day			
12					12	off day			
	off day				13	off day			
	off day				14	off day			
15					**15**				
	off day				**16**				
	off day				**17**				
	off day				**18**				
	off day				**19**				
	off day				**20**				
	off day				**21**				

	off day				22	off day			
	off day				23	off day			
	off day				24	off day			
	off day				25	off day			
26					26	off day			
	off day				27	off day			
	off day				28	off day			

My signature signifies that the study drug(s) have been taken as indicated:
Patient's Signature _____Date: _____

Dose Dense Arm

Cycle 2 and beyond

Patient Initials: _____Patient ID#: _____Phase____/Cycle #_____

_____

This diary is for you to record that you took the drugs as instructed by your doctor. Please put a check mark or your initials after each dose. Please sign this diary at the end of the cycle. Bring the diary and all study drug bottles back to your next clinic visit.

TG02 AM **Zotiraciclib** **Frequency:** Only on Day 1, Day 12, Day 15, Day 26 *Cycle 1 only: extra dose on D -3					**Temozolomide** PM **Frequency**: 7days ON, 7 days OFF				
DAY #	**DATE**	**TIME**	**DOSE**	**INITIALS**	**DAY #**	**DATE**	**TIME**	**DOSE**	**INITIALS**
1					**1**				
	off day				**2**				
	off day				**3**				
	off day				**4**				
	off day				**5**				
	off day				**6**				
	off day				**7**				
	off day				8	off day			
	off day				9	off day			
	off day				10	off day			
	off day				11	off day			
12					12	off day			
	off day				13	off day			
	off day				14	off day			
15					**15**				
	off day				**16**				
	off day				**17**				
	off day				**18**				
	off day				**19**				
	off day				**20**				
	off day				**21**				
	off day				22	off day			
	off day				23	off day			
	off day				24	off day			
	off day				25	off day			
26					26	off day			

	off day				27	off day			
	off day				28	off day			

My signature signifies that the study drug(s) have been taken as indicated:
Patient's Signature _____Date: _____

Metronomic arm

Cycle 1

Patient Initials: _____Patient ID#: _____Phase___/Cycle # 1 _____

This diary is for you to record that you took the drugs as instructed by your doctor. Please put a check mark or your initials after each dose. Please sign this diary at the end of the cycle. Bring the diary and all study drug bottles back to your next clinic visit.

TG02 AM **Zotiraciclib** **Frequency:** Only on Day 1, Day 12, Day 15, Day 26 *Cycle 1 only: extra dose on D -3					**Temozolomide** PM **Frequency**: Daily				
DAY #	**DATE**	**TIME**	**DOSE**	**INITIALS**	**DAY #**	**DATE**	**TIME**	**DOSE**	**INITIALS**
-3 *									
	off day								
	off day								
1					**1**				
	off day				**2**				
	off day				**3**				
	off day				**4**				
	off day				**5**				
	off day				**6**				
	off day				**7**				
	off day				**8**				
	off day				**9**				
	off day				**10**				
	off day				**11**				
12					**12**				
	off day				**13**				
	off day				**14**				
15					**15**				
	off day				**16**				
	off day				**17**				
	off day				**18**				
	off day				**19**				
	off day				**20**				
	off day				**21**				
	off day				**22**				
	off day				**23**				
	off day				**24**				
	off day				**25**				
26					**26**				
	off day				**27**				

	off day				**28**				

My signature signifies that the study drug(s) have been taken as indicated:
Patient's Signature _____Date: _____

Metronomic Arm

Cycle 2 and beyond

Patient Initials: _____Patient ID#: _____Phase___/Cycle #_____

This diary is for you to record that you took the drugs as instructed by your doctor. Please put a check mark or your initials after each dose. Please sign this diary at the end of the cycle. Bring the diary and all study drug bottles back to your next clinic visit.

TG02 AM **Zotiraciclib** **Frequency:** Only on Day 1, Day 12, Day 15, Day 26 *Cycle 1 only: extra dose on D -3					**Temozolomide** PM **Frequency**: Daily				
DAY #	**DATE**	**TIME**	**DOSE**	**INITIALS**	**DAY #**	**DATE**	**TIME**	**DOSE**	**INITIALS**
1					**1**				
	off day				**2**				
	off day				**3**				
	off day				**4**				
	off day				**5**				
	off day				**6**				
	off day				**7**				
	off day				**8**				
	off day				**9**				
	off day				**10**				
	off day				**11**				
12					**12**				
	off day				**13**				
	off day				**14**				
15					**15**				
	off day				**16**				
	off day				**17**				
	off day				**18**				
	off day				**19**				
	off day				**20**				
	off day				**21**				
	off day				**22**				
	off day				**23**				
	off day				**24**				
	off day				**25**				
26					**26**				

	off day				**27**				
	off day				**28**				

My signature signifies that the study drug(s) have been taken as indicated:
Patient's Signature _____Date: _____