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**TITLE:** A Phase I Trial Combining Triapine with Radiation Therapy for Recurrent Glioblastoma

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**NCI-Supplied Agent:** Triapine (NSC 663249)

**IND #:** TBD

**IND Sponsor:** NCI DCTD

**Protocol Type / Version # / Version Date:** Original / September 20, 2024

**SCHEMA**



|  |
| --- |
| **Dose Escalation Schedule** |
| **Dose Level** | **Dose\*** |
| **Triapine****(mg)\*\*** | **Radiation** |
| Level 1 (starting) | 50 | 35Gy in 10 fractions |
| Level 2 | 100 | 35Gy in 10 fractions |
| Level 3 | 150 | 35Gy in 10 fractions |
| Level 4 | 200 | 35Gy in 10 fractions |
| *\*Doses are stated as exact dose in units (*e.g.*, mg/m2, mcg/kg,* etc.*) rather than as a percentage.**\*\*Doses are administered once daily within 2 hours prior to radiation treatments.* |

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# OBJECTIVES

## Primary Objectives

* + 1. To identify the safety and maximally tolerated dose (MTD) of oral triapine used in combination with radiation therapy for patients with recurrent glioblastoma (GBM).

## Secondary Objectives

* + 1. To observe and record anti-tumor activity. Although the clinical benefit of this drug has not yet been established, the intent of offering this treatment is to provide a possible therapeutic benefit, and thus the patient will be carefully monitored for tumor response and symptom relief in addition to safety and tolerability.
		2. To determine the pharmacokinetics of oral triapine in plasma and the central nervous system (CNS).
		3. To evaluate the efficacy of triapine when administered in combination with radiation therapy by assessing:
* Progression-free survival (PFS)
* Overall survival (OS)
* The proportion of patients requiring bevacizumab for symptom control
* The correlation of genetic mutations in select genes (*e.g., p53*, *p16*, *KRAS*, and *Pi3k/mTOR/AKT*) with tumor response and clinical outcomes

# BACKGROUND

## Study Disease(s)

Glioblastoma (GBM) is an aggressive malignancy that accounts for 48% of malignant central nervous system tumors (Koshy *et al.,* 2012). For the past two decades, despite administration of standard-of-care therapy, comprising maximal safe resection followed by concurrent radiation and temozolomide, adjuvant temozolomide, and tumor treating fields (TTF), there have been minimal improvement in survival rates (Herrlinger *et al.,* 2019; Stupp *et al.,* 2005; Stupp *et al.,* 2017). As a result, the median survival remains 14-20 months. Approximately 80% of treatment failures occur within the radiation treatment field, implying the presence of radioresistant clones. Radiation dose escalation studies beyond 60Gy, with or without concurrent chemotherapy, have not improved survival or local disease control, but have increased risk for radiation necrosis (Baumert *et al.,* 2008; Cardinale *et al.,* 2006; Chan *et al.,* 2002; Chang *et al.,* 1983; Corn *et al.,* 2009; Lawrence *et al.,* 2010; Nelson *et al.,* 1988; Prados *et al.,* 2001; Souhami *et al.,* 2004; Tsien *et al.,* 2009).

While there is no consensus for the optimal treatment for recurrent GBM, secondary analyses indicate that patients who undergo salvage therapies may experience better survival, neurologic symptoms, and quality-of-life compared to patients who did not receive such interventions (Scoccianti, *et al.,* 2018; Shi *et al.,* 2018). Re-irradiation is one treatment option for select patients with limited-volume recurrent GBM (Fogh *et al.,* 2010; Grosu *et al.,* 2005; Lederman *et al.,* 2000). Historically, 6-month PFS was 15%, prompting many clinical trials to seek a 13-15% improvement in outcomes (Minniti *et al.,* 2015; Reardon *et al.,* 2012). In a recent meta- analysis, re-irradiation resulted in a 6-month PFS rate of 43% and 1-year OS of 36% (Kazmi *et al.,* 2019). NRG/RTOG 1205 reported that re-irradiation (35Gy in 10 fractions) in addition to bevacizumab significantly improved median PFS from 3.8 months (bevacizumab alone) to 7.1 months (hazards ratio [HR] 0.73, [95% CI, 0.53-1]) and was well tolerated (Tsien *et al.,* 2023). Bevacizumab was selected as the control arm based on EORTC 26101, which led to its Food and Drug Administration (FDA) approval for recurrent GBM. EORTC 26101 demonstrated that combining bevacizumab with lomustine significantly improved median PFS compared to lomustine alone, but there was no significant improvement in OS (Wick *et al.,* 2017). Similarly, the combination of re-irradiation and bevacizumab arm in NRG/RTOG 1205 did not improve OS, consistent with outcomes from numerous other trials focusing on recurrent GBM. These findings underscore that radiation alone is not sufficient to improve oncologic outcomes for patients with GBM.

The presence of significant genetic heterogeneity and alterations in key signaling pathways within the tumor contribute to the development of aggressive, treatment-resistant phenotypes (Matthews *et al.,* 2022; McLendon *et al.,* 2008; Patel *et al.,* 2014; Snuderl *et al.,* 2011). Heterogeneity results from uncontrolled, error-prone DNA replication and repair. These processes are influenced by a sustained yet unbalanced supply of deoxyribonucleotide triphosphates (dNTPs), the essential building blocks of DNA (Bester *et al.,* 2011; Chabes *et al.,* 2003; Saxena and Zou, 2022; Weinberg *et al.,* 1981). Ribonucleotide reductase (RNR) is a rate-limiting enzyme that mediates the production of dNTPs (Fairman *et al.,*2011). RNR is composed of two non-identical regulatory subunits, with the second subunit (Regulatory subunit 2 [RRM2]) controlling RNR expression and activity (Thelander and Reichard, 1979). RRM2 contains a di-iron center that catalyzes the reduction of 2’ carbon of ribonucleotides and is critical to its function (Aye *et al.,* 2015; Cotruvo and Stubbe, 2011). RRM2 is a target of many signaling pathways frequently dysregulated in cancer such as KRAS, p53, and mTOR, and has been reported to be upregulated in several cancer types (He *et al*., 2017; Shen *et al.,* 2007; Yoshida *et al.*, 2011). These findings suggest an important role of RRM2-mediated dNTP metabolism in treatment resistance which makes RRM2 an attractive therapeutic target in GBM.

## CTEP IND Agent (Triapine)

Triapine belongs to the class of iron free radical site (M2)-affecting RNR inhibitors that are derivatives of a-heterocyclic carboxaldehyde thiosemicarbazone (HCT), which is 65-5,000 times more potent than the only clinically approved M2-affecting RNR inhibitor, hydroxyurea (Investigator’s Brochure, 2018). Triapine was designed to avoid the metabolic fate of the initial lead structure in the HCT series, which underwent rapid metabolism and had a short biological half-life of 2.5-10 minutes in patients.

* + 1. Nonclinical Summary

Triapine Injection has been shown to inhibit RNR (as measured by cytosine diphosphate [CDP] reductase activity) *in vitro*, with an average IC50 value of 0.3 mcM against a highly purified enzyme and 0.82 mcM against a partially purified enzyme, from the Ehrlich carcinoma (Investigator’s Brochure, 2018). Triapine Injection has also shown anti-tumor activity against M109 and L1210 tumors, and the HU-resistant subline version of L1210 tumors. The average IC50 values for the anti-tumor activities were 0.75-1.6 mcM. Triapine inhibited CDP reductase activity for a longer time in tumor cells as compared to normal cells, where the R50 (the time for 50% recovery from Triapine-induced inhibition of DNA synthesis) was 10.1 hours in L1210 tumors cells but only 4.8-7.3 hours in normal cells.

In mouse studies, when mice were injected intraperitoneally twice daily for six consecutive days at doses of 10-30 mg/kg/injection, triapine induced a “bell-shaped” dose response curve, where the maximum anti-tumor effect was observed at 15 mg/kg/injection, with a treatment/control survival ratio of 262% (Investigator’s Brochure, 2018). Triapine has also been shown to reduce tumor size in mice previously implanted at a stringent distal site with murine M109 lung carcinoma and the human A2780 ovarian carcinoma. When combined with a DNA-damaging agent such as cisplatin, triapine has been shown to induce an at least additive anti-tumor effect in mice with L1210 leukemia, with an enhanced prolongation of survival time beyond the time induced by either agent alone. Conversely, no enhancement in prolongation of survival time was found when triapine was combined with the nucleoside analogs gemcitabine or cytosine arabinoside, indicating that triapine may inhibit the DNA repair process.

The pharmacological effects of triapine on the central nervous system, inflammatory responses, cholesterol metabolism, gastrointestinal (GI) tract, cardiovascular system, and immune system were examined in rodents (Investigator’s Brochure, 2018). Triapine only affected the GI tract and cholesterol metabolism, eliminating aspirin-induced ulcers and selectively and significantly reducing average serum cholesterol and low-density lipoprotein. The serum pharmacokinetic (PK) parameters of triapine after intravenous (IV) administration were evaluated in rats and dogs, where generally the concentration-time profile was biphasic. Rate of administration was not found to affect the distribution and metabolism of triapine in dogs, nor does triapine bioaccumulate in dogs with single daily doses for up to five days. A study on the distribution and elimination of triapine was conducted in rats, where the primary route of excretion was feces, suggesting the involvement of biliary excretion. Data suggested an even distribution of triapine between intra- and extra-cellular space, with widespread distribution to all organs except those related to the CNS.

The acute and sub-chronic toxicity of triapine was evaluated in dogs and in rats (Investigator’s Brochure, 2018). In dogs, no treatment-related effects were observed at 1 mg/kg of triapine when administered by IV over 15 minutes once daily for five consecutive days. When administered at 3 mg/kg over 15 or 120 minutes for one day or five consecutive days, triapine induced mild toxicological effects that were indirectly related to the rate of administration. In rats, an injection of triapine administered once daily for five consecutive days produced a wide range of toxicity; the broad spectrum of toxicological effects was consistent with the RNR inhibitory properties of triapine. The following effects were related to triapine: mortality, reductions in total protein and albumin levels, reductions in red and white blood cell counts, reductions in platelet counts, reductions in body and thymic weights, increases in the liver and lung weights with corresponding microscopic findings, and microscopic findings of the adrenal cortex, testes, bone marrow, and kidney. The intensity of most of these effects were dose-related and essentially all effects induced by triapine were reversible. Mutagenicity assays indicated that triapine did not induce any gene mutations.

* + 1. Clinical Summary

Triapine has previously been shown to be safe in several phase 1 and 2 trials as a radiosensitizer alone or in combination with chemotherapy in several cancer types (Kunos *et al.,* 2017). In a phase 1 dose-finding study of IV triapine and cisplatin in patients with advanced-stage malignancies, the MTD of triapine was 96mg/m2 daily days 1-4 when given with 75mg/m2 cisplatin. The most frequent grade 3-4 toxicities observed were leukopenia and thrombocytopenia. Pharmacokinetic data indicate oral triapine bioavailability of 88%. Chao et al. (2012) conducted a phase 1 trial of oral triapine in patients with advanced-stage solid tumors. The MTD was 150mg every 12 hours on days 1-3, 8-10, and 15-17 of every 28-day cycle. Triapine was well tolerated, and common toxicities were grade 1-2 (reversible) hematologic toxicities, fatigue, hypoxia, nausea, and hyperglycemia. The oral bioavailability of triapine was 67% ± 29%. The MTD by oral route was 33% greater than IV route (Kunos *et al.,* 2017). Unpublished data from the University of Pittsburg show triapine concentrations peaked in plasma two hours after oral administration.

Triapine has shown promising activity in pancreatic cancer. In a phase 1 dose escalation study of IV triapine co-administered with radiation therapy (50.4Gy) for locally advanced pancreatic cancer, the MTD was not reached (Martin *et al.,* 2012). Thus, combining triapine at 72mg/ m2 given three times weekly with conventionally fractionated radiation was well tolerated. Ninety-two percent of patients experienced freedom from local tumor progression, suggesting highly promising results in a disease where local failure occurs in 50-70% of patients after chemoradiation. Mackenzie et al. (2007) conducted a phase II trial of IV triapine (105mg/ m2) concurrently with gemcitabine (1,000 mg/ m2) in locally advanced pancreatic cancer patients. Both agents were given on days 1, 8, and 15 of a 28-day cycle. Of 26 patients, 11 patients had stable disease. Median survival was 9 months with a 1-year OS of 28%. Combination triapine and gemcitabine was well tolerated, with common non-hematologic toxicities including hypoxia due to methemoglobinemia, hyperglycemia, elevated liver enzymes, and fatigue.

Finally, triapine was extensively tested in patients with stage IB2-IVB cervical and stage II-IV vaginal cancers. In a phase 2 study evaluating IV triapine (25mg/ m2) delivered three times weekly (days 1,3, 5) concurrently with once weekly IV cisplatin (40mg/ m2) and daily conventionally fractionated pelvic radiation (45Gy total), triapine achieved clinical response in 96% (24/25) of patients (95% CI, 80-99%) after median follow-up of 20 months. The most frequent toxicities observed were fatigue, nausea, diarrhea, and reversible hematological and electrolyte abnormalities. Based on 29 eligible patients from phase 1 and 2 trials of combination triapine and chemoradiotherapy, OS was 83% (95% CI, 63- 92%) at 30 months and 59% (95% CI, 39-74%) at 60 months (Kunos and Ivy, 2018). As a comparison, OS estimates at 30 and 60 months with cisplatin-based chemoradiation alone were 70% and 30%, respectively (Rose *et al.,* 2007). Despite promising phase 2 results, NRG-GY006 phase 3 trial failed to demonstrate improvement in OS with the addition of triapine to chemoradiation for locally advanced cervical and vaginal cancer. The study was designed to detect a 10% improvement in 3-year OS with addition of triapine compared to chemoradiation alone with 80% power and 2.5% significance level including one interim futility analysis (Leath *et al.,* 2023). Seventy-six percent of patients completed the entire protocol directed therapy. Triapine was well tolerated, and there was no difference in severe grade 3-5 toxicities between the two arms. Median follow-up was 28 months (IQR, 15-45). Median PFS and OS were not reached in both arms, with hazard rate ratio for death 1.018 (95% CI, 0.634-1.635). Despite the ultimate lack of improvement in OS in NRG-GY006, triapine remains worthy of exploration for GBM for two main reasons:

1. There are distinct underlying biological characteristics between GBM and HPV-driven gynecological cancers.
2. Triapine combined with standard-of-care cisplatin for cervical cancer has not shown substantial clinical activity together (Feun *et al.,* 2002; Kunos *et al.,* 2017; Wadler *et al.,* 2004) while recent pre-clinical studies specific to GBM suggest that triapine may independently overcome resistance to both radiation and temozolomide (Corrales-Guerrero *et al.,* 2023; Perrault *et al.,* 2023).

## Rationale

Currently, there is a clear lack of effective interventions that overcome genetic heterogeneity and the resulting treatment resistance. There is a critical need to test candidate agents that may potentially overcome genetic heterogeneity and restore tumor response to radiation. Without such information, the promise of improving disease control and survival for the treatment of GBM will remain unrealized. 3-aminopyridine-2-carboxyaldehyde thiosemicarbazone (3-AP or triapine) is a competitive iron inhibitor that blocks the essential di-iron center of RRM2 and holds potential to overcome genetic heterogeneity. By inhibiting the function of dysregulated RRM2 in cancer cells, triapine can influence RNR to restore greater genomic stability (Cotruvo and Stubbe, 2011; Zhan *et al.,* 2021). Triapine is also a small molecule able to pass through the blood-brain barrier, a well-known issue preventing many therapeutic agents from entering the central nervous system (Finch *et al.,* 2000; Jiang *et al.,* 2006). Our long-term goal is to integrate therapeutic interventions that would overcome treatment resistance in GBM.

Phase 1 and 2 trials investigating the use of triapine suggest it is well tolerated, with main toxicities including hypoxia due to methemoglobinemia, reversible hematologic and electrolyte abnormalities, hyperglycemia, and fatigue. Favorable disease responses have been observed particularly for cervical, vaginal, and pancreatic cancers. We hypothesize that inhibiting RRM2 activity with triapine in combination with radiation will not only be well tolerated, but also enhance disease control for patients with GBM. This hypothesis has been formulated largely based on our preliminary data generated using well-characterized human cancer cell lines and *in vivo* human xenograft mouse models. The rationale for this project is that a determination of safety, pharmacokinetics in the central nervous system, and therapeutic efficacy of triapine can offer strong evidence where new strategies for the treatment of GBM can be developed. As a pivotal first step towards our long-term goal, we propose an initial phase 1 dose escalation study to evaluate the safety of triapine and radiation for patients with recurrent GBM. The results from our primary and secondary objectives from this trial, alongside preclinical data from Perrault et al. (2023) are expected to have an important impact in providing evidence for the design of future clinical trials of triplet combination therapy (triapine, radiation, and temozolomide), ultimately providing a new opportunity to improve survival for patients with newly diagnosed GBM.

In our preclinical studies, described further in this proposal, we demonstrated that targeting RRM2 with triapine both *in vitro* and *in vivo* sensitized GBM cells to ionizing radiation*,* leading to greater radiation damage, local tumor control, and survival in mice (Corrales-Guerrero *et al.,* 2023). Furthermore, Perrault et al. (2023) showed that inhibiting RRM2 activity with triapine also significantly increased anti-tumor activity of temozolomide *in vivo*. Altogether, triapine may overcome treatment resistance in GBM.

We recently published pre-clinical data that demonstrate triapine could effectively radiosensitize GBM cell lines (Corrales-Guerrero *et al.,* 2023). First, we found that *RRM2* expression was dramatically increased in GBM. When comparing *RRM2* expression levels across 33 cancer types from The Cancer Genome Atlas (TCGA) relative to normal tissue, the highest fold increase was found in GBM (**Figure 1A**). Furthermore, *RRM2* expression levels significantly increased with histologic tumor grade across multiple public datasets, indicating more aggressive histologic grade is correlated with RRM2 expression **(**TCGA data shown in **Figure 1B**). In addition, higher RRM2 expression was noted in isocitrate dehydrogenase (IDH) wild-type gliomas **(Figure 1C**). Elevated *RRM2* expression was significantly associated with worse OS in two glioma datasets (Chinese Glioma Genome Atlas [CGGA] and RecuR) shown in **Figure 1D-E**. Furthermore, uni- and multivariable analyses found *RRM2* expression to be significantly associated with poor survival. Altogether, RRM2 plays a role in sustaining aggressive forms of glioma and could serve as a potential therapeutic target.



**Figure 1. RRM2 expression is elevated in GBM and associated with worse OS.** (A) RRM2 mRNA expression in The Cancer Genome Atlas (TGCA) GBM samples compared to normal tissue. (B) *RRM2* expression by histologic tumor grade across all cancer types in TGCA. (C) *RRM2* expression by isocitrate dehydrogenase (IDH) status. (D) OS from CGGA study cohort by *RRM2* expression levels. (E) OS from RecuR study cohort by *RRM2* expression levels. p<0.05: \*, p<0.01: \*\*, p<0.001: \*\*\*, p<0.0001: \*\*\*\* (Corrales-Guerrero *et al.,* 2023).

Radiation clonogenic assays were performed using LN229 and U87MG/EGFRvIII GBM cells lines to test the radiosensitizing effect of triapine (Corrales-Guerrero *et al.,* 2023). Cell lines were treated with triapine (1mcM) or control (DMSO) for 24 hours and subjected to increasing doses of radiation (0,2,4,6,8Gy). The dose enhancement ratio (DER) was calculated based on the ratio between the effects on each cell line’s viability compared to the control. A DER greater than 1.2 is generally considered clinically significant. Triapine sensitized LN229 and U87MG/EGFRvIII cells lines with DERs of 1.89 and 1.25, respectively (**Figure 2A-B**). Similar effects were observed in an ionizing radiation-resistant (IRR)-08-387 patient-derived primary GBM cell line which resulted in a DER of 1.41 (**Figure 2C**). Additionally, triapine enhanced radiation-induced DNA damage in LN229 and U87MG/EGFRvIII GBM cell lines shown by persistently elevated γH2AX levels and nuclear foci counts 24 hours after radiation (**Figure 2D-G**).



**Figure 2. RRM2 inhibitor, triapine, sensitizes GBM cells to radiation.** Radiation clonogenic assays are shown for (A) LN229, (B) U87MG/EGFRvIII, and (C) IRR-08-387 cell lines. (D-E) Western blotting for γH2AX from LN229 and U87MG/EGFRvIII cells treated with control (DMSO) or triapine and harvested before or up to 24 hours after irradiation. (F-G) Quantification of γH2AX foci observed in LN229 U87MG/EGFRvIII cells treated with control or triapine after irradiation using immunofluorescence. p<0.05: \*, p<0.01: \*\*, p<0.001: \*\*\*, p<0.0001: \*\*\*\* (Corrales-Guerrero *et al.,* 2023).

Two sets of *in vivo* experiments were conducted, both demonstrating that combination triapine and radiation led to the largest reduction in tumor growth (Corrales-Guerrero *et al.,* 2023). In the first set, U87MGEGFRvIII cells were subcutaneously injected into the flanks of athymic nude mice. Once tumors reached 100-200 mm3, mice were randomized into one of four groups: control, triapine alone, radiation alone, or combination triapine and radiation. In the groups receiving radiation, 4Gy per day was delivered for 5 consecutive days (total of 20Gy). Triapine (10mg/kg) was injected intraperitoneally 2 hours prior to radiation therapy. Tumor size was measured at least twice per week using digital calipers. **Figure 3A-B** shows combination triapine and radiation resulted in the longest delay in tumor growth and survival. Median survival was 10 (control), 9 (triapine alone), 26 (radiation alone), and 37.5 days (combination triapine + radiation, p<0.05).



**Figure 3. Triapine inhibition of RRM2 sensitizes GBM U87MG/EGFRvIII cells to radiation *in vivo*.** (A) Individual tumor size was tracked for the duration of the experiment and normalized to the volume measured at the beginning of the treatment. (B) Kaplan-Meier survival curves showing OS of mice by treatment groups. \*\*Statistical comparison between the irradiation (IR) group and combination triapine + IR group using log-rank test was statistically significant (p=0.0086) (Corrales-Guerrero *et al.,* 2023).

In the second set of unpublished *in vivo* experiments with an orthotopic model, KR158B-luciferase bearing syngeneic GBM cells (Kluc 2 × 105 in 2 μL 1xPBS) were implanted into the brains of immunocompetent C57Bl/6 mice using stereotactic localization. Seven days after implantation, the mice were randomized into one of the four treatment groups as described in the first set of *in vivo* experiments. In the groups receiving radiation, 2Gy per day was delivered for 2 consecutive days (total of 4 Gy). Triapine (10 mg/kg) was injected intraperitoneally 2 hours prior to radiation therapy. Luciferin solution (100 mg/kg) was used for imaging after tumor formation, and IVIS Lumina II imaging platform was used to detect and quantify the signal. Combination triapine and radiation again led to the largest decrease in luciferase signal by day 21, reflecting the longest tumor growth delay (**Figure 4A**). The combination treatment group also significantly prolonged OS **(Figure 4B**).



**Figure 4. Triapine inhibition of RRM2 sensitizes GBM cells to radiation in intracranial xenograft mouse model.** (A) Luciferase signal after 21 days by treatment group. (B) Survival of mice by treatment groups.

Taken together, we observe that triapine has potent radiosensitizing ability in GBM models, leading to enhanced tumor response and improved survival to radiation therapy both *in vitro* and *in vivo* relative to radiation alone. Therefore, we believe triapine is worthy of further clinical exploration for the treatment of GBM.

## Correlative Studies Background

* + 1. **IDH1/2**
			1. Biologic rationale: IDH-mutant astrocytic gliomas are considered biologically and prognostically distinct from IDH-wildtype glioblastoma. IDH1/2 mutations in high-grade gliomas have been recognized as a favorable prognostic factor and are associated with favorable disease-free and OS.
			2. Hypothesis: We hypothesize that IDH1/2-mutant gliomas will have favorable clinical outcomes following triapine and re-irradiation for recurrent high-grade astrocytic gliomas.
			3. Relevant preclinical data: In a prospective translational study of 301 patients with glioblastoma in the German Glioma Network, IDH-mutations were associated with significantly prolonged progression-free survival and trend towards prolonged OS (Weller *et al.*, 2009). Furthermore, the presence of IDH1 mutation was associated with significantly improved survival in all glioma grades and confirmed to be an independent favorable prognostic marker on multivariate analysis (Sanson *et al.*, 2009).
		2. **MGMT Methylation Status**
			1. Biologic rationale: O6-methylguanine-methyltransferase (MGMT) promoter methylation has been considered a positive prognostic factor for glioblastoma and high-grade astrocytic tumors.
			2. Hypothesis: We hypothesize that MGMT-promotor methylated gliomas will have favorable clinical outcomes following triapine and re-irradiation for recurrent high-grade astrocytic gliomas.
			3. Relevant preclinical data: EORTC 26981-2298 / NCIC trial (*i.e*., the Stupp trial) demonstrated that MGMT-promoter methylation was the strongest predictor of survival, regardless of whether glioblastoma patients were treated with temozolomide plus radiation therapy or radiation therapy alone (Stupp *et al.*, 2009). RTOG 0525 also provided clear evidence of the positive prognostic and predictive role of MGMT-promoter methylation as it was associated with significantly improved survival and treatment response ([Gilbert](https://doi.org/10.1200/JCO.2013.49.6968) *et al.*, 2013).
		3. **Whole Exome Sequencing (Tissue- and Blood-based)**
			1. Biologic rationale: RRM2 is a target of many signaling pathways frequently dysregulated in cancer such as *TP53, KRAS,* and *mTOR*. However, it is currently unknown if key genes along these pathways are potential predictive biomarkers of response to treatment with triapine combined with radiation therapy.
			2. Hypothesis: We hypothesize there is an association with genetic mutations along *TP53, KRAS,* and *mTOR pathways* with tumor response and clinical outcomes.
			3. Relevant preclinical data: Balanced deoxyribonucleotide pools are essential for genomic stability and cell survival. *P53* has demonstrated to suppress RNR and RRM2 via inhibiting mammalian target of rapamycin complex 1 (mTORC1) (He *et al.*, 2017; Shen *et al.*, 2017). *KRAS* has been associated with upregulation of RRM2 in colorectal cell lines (Yoshida *et al.*, 2011).
		4. **RNAseq**
			1. Biologic rationale: RRM2 is dysregulated in many cancers and elevated in glioblastoma. Therefore, RRM2 makes an attractive therapeutic target in glioblastoma. Triapine targets and inhibits RRM2, which holds potential to overcome genetic heterogeneity and restore tumor response to radiation. However, it is currently unknown whether RRM2 expression levels in humans are predictive biomarkers of response to treatment with triapine combined with radiation therapy.
			2. Hypothesis: We hypothesize there is an association of RRM2 expression and other molecular signatures for radiosensitivity with tumor response and clinical outcomes after treatment with triapine and re-irradiation.
			3. Relevant preclinical data: RRM2 expression is increased in GBM, and higher levels are associated with higher histologic tumor grade and among IDH-wildtype glioblastomas. Furthermore, RRM2 expression has been associated with poor survival in two large glioma datasets (Corrales-Guerrero *et al.*, 2023). Pre-clinical work from Williams Lab demonstrated that inhibiting RRM2 with triapine enhanced radiation-induced DNA damage in glioma cell lines and intracranial xenograft mouse models (Corrales-Guerrero *et al.*, 2023).
		5. **Triapine Pharmacokinetics (PK) (Blood-based)**
			1. Biologic rationale: To confirm oral triapine at the proposed dose levels achieve comparable serum concentrations as historic controls in advanced-stage solid cancers.
			2. Hypothesis: We hypothesize the pharmacokinetics of oral triapine is comparable to historical controls.
			3. Relevant preclinical data: Pharmacokinetic data show that exposure of orally administered triapine increased in a dose-dependent manner (Chao *et al.*, 2011). Triapine in this study was given once every 12 hours for 5 consecutive doses on days 1-3, 8-10, and 15-17 of every 28-day cycle starting at 50mg dose. The maximum-tolerated dose was 150mg per dose on this dosing schedule. Peak concentrations occurred at approximately the same time when the drug was administered orally. Mean oral bioavailability (Foral/IV) across all dose levels was 0.69 ± 0.29. At an oral dose of 150mg (the maximally tolerated dose), the mean AUC was 10.6 ± 7.6 mcMh, and mean peak serum concentration was 5 ± 3.8 mcM.
		6. **Triapine PK (Cerebrospinal fluid [CSF]-based)**
			1. Biologic rationale: To determine whether triapine crosses the human blood-brain barrier.
			2. Hypothesis: We hypothesize that triapine crosses the human blood-brain barrier.
			3. Relevant preclinical data: Preclinical investigations performed in mice demonstrate triapine can cross the blood-brain barrier and is effective in killing leukemic cells in the brain (Avery *et al.*, 1990). Triapine has also been shown to be a highly hydrophobic small molecule with ability to achieve sufficient concentrations in the brain to elicit promising neuroprotectant effects (Jiang *et al.*, 2006).

# PATIENT SELECTION

## Eligibility Criteria

* + 1. Patients must have histologically, molecularly, or cytologically confirmed GBM or its variants, astrocytic tumor, or other adult-type diffuse glioma that is recurrent for which standard curative measures do not exist or are no longer effective.

Patients originally diagnosed with a lower-grade glioma and subsequent diagnosis of GBM (or variants) are eligible.

* + 1. Tumors ≤6 cm in maximal diameter.
		2. Patients with no known G6PD deficiency may be in this study. Further testing for G6PD deficiency will not be required.
		3. Patients must have at least a 6-month break from last dose of radiation therapy.

Re-irradiation within 6 months may increase risk for radiation necrosis/edema, which will affect toxicity assessment and patient safety. Additionally, GBM and other high-grade astrocytic tumors can exhibit pseudo-progression within 6 months from completing definitive, 1st line radiation therapy, and re-irradiation during this period will increase risk for misattribution of effect.

* + 1. Prior history of standard dose radiation for gliomas of 59.4-60 Gy in 1.8-2 Gy per fraction (or equivalent or lower) is allowed.
		2. Patients who received non-standard radiation such as stereotactic radiosurgery are eligible as long as there is:
1. A new tumor outside the original radiotherapy field as determined by the investigator.
2. There is histologic confirmation of tumor on biopsy or resection.
3. Imaging findings on MRI spectroscopy/perfusion imaging/nuclear medicine imaging are consistent with true progressive disease.
4. Approved by the principal investigator.
	* 1. Age ≥18 years.

Because no dosing or adverse event data are currently available on the use of triapine in patients <18 years of age, children are excluded from this study.

* + 1. ECOG performance status ≤2 (Karnofsky ≥60%, see [Appendix A](#_APPENDIX_A_PERFORMANCE)).
		2. Patients must have adequate organ and marrow function as defined below:
* absolute neutrophil count ≥1,500/mcL
* hemoglobin ≥8 g/dL
* platelets ≥100,000/mcL
* total bilirubin ≤1.5 × institutional upper limit of normal (ULN)
* AST(SGOT)/ALT(SGPT) ≤2.5 × institutional ULN
* creatinine ≤1.5 x ULN

OR

* glomerular filtration rate (GFR) ≥50 mL/min/1.73 m2 (see [Appendix B](#_APPENDIX_B_FORMULA))
	+ 1. Human immunodeficiency virus (HIV)-infected patients on effective anti-retroviral therapy with undetectable viral load within 6 months are eligible for this trial.
		2. For patients with evidence of chronic hepatitis B virus (HBV) infection, the HBV viral load must be undetectable on suppressive therapy, if indicated.
		3. Patients with a history of hepatitis C virus (HCV) infection must have been treated and cured. For patients with HCV infection who are currently on treatment, they are eligible if they have an undetectable HCV viral load.
		4. Patients with a prior or concurrent malignancy whose natural history or treatment does not have the potential to interfere with the safety or efficacy assessment of the investigational regimen are eligible for this trial.
		5. Patients with known history or current symptoms of cardiac disease, or history of treatment with cardiotoxic agents, should have a clinical risk assessment of cardiac function using the New York Heart Association Functional Classification. To be eligible for this trial, patients should be class II or better.
		6. Patients must be able to swallow whole capsules.
		7. Patients must be able to undergo MRIs. Patients with non-compatible devices with MRI can be eligible if CT scans of sufficient quality are obtained. However, patients without non-compatible devices may not use CT scans to meet this requirement*.*
		8. The effects of triapineon the developing human fetus are unknown. For this reason and becauseRNR inhibitor agent and radiation are known to be teratogenic, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry, for the duration of study participation, and for 12 months after finishing study treatment. People of child-bearing potential must have a negative serum or urine pregnancy test (minimum sensitivity 25 IU/L or equivalent units of HCG) within 2 weeks of registration. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately. Men treated or enrolled on this protocol must also agree to use adequate contraception prior to the study, for the duration of study participation, and 12 months after completion of triapineadministration.
		9. Ability to understand and the willingness to sign a written informed consent document. Legally authorized representatives may sign and give informed consent on behalf of study participants.

## Exclusion Criteria

* + 1. Patients who have not recovered from adverse events due to prior anti-cancer therapy (*i.e.*, have residual toxicities > Grade 1) with the exception of alopecia.
		2. Patients who are receiving any other investigational agents.
		3. Patients who are actively taking medications that are known to induce methemoglobinemia.
		4. History of allergic reactions attributed to compounds of similar chemical or biologic composition to triapine.
		5. Patients with uncontrolled intercurrent illness, active infections, or any other significant condition(s) that would make participation in this protocol unreasonably hazardous.
		6. Pregnant women are excluded from this study because triapine is aRNR inhibitor agent with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with triapine*,* breastfeeding should be discontinued if the mother is treated withtriapine. These potential risks may also apply to the radiation used in this study.

## Inclusion of Women and Minorities

NIH policy requires that women and members of minority groups and their subpopulations be included in all NIH-supported biomedical and behavioral research projects involving NIH-defined clinical research unless a clear and compelling rationale and justification establishes to the satisfaction of the funding Institute & Center (IC) Director that inclusion is inappropriate with respect to the health of the subjects or the purpose of the research. Exclusion under other circumstances must be designated by the Director, NIH, upon the recommendation of an IC Director based on a compelling rationale and justification. Cost is not an acceptable reason for exclusion except when the study would duplicate data from other sources. Women of childbearing potential should not be routinely excluded from participation in clinical research. Please see <http://grants.nih.gov/grants/funding/phs398/phs398.pdf>.

# REGISTRATION PROCEDURES

## Investigator and Research Associate Registration with CTEP

Food and Drug Administration (FDA) regulations require sponsors to select qualified investigators. National Cancer Institute (NCI) policy requires all individuals contributing to NCI-sponsored trials to register with their qualifications and credentials and to renew their registration annually. To register, all individuals must obtain Cancer Therapy Evaluation Program (CTEP) credentials necessary to access secure NCI Clinical Oncology Research Enterprise (CORE) systems. Investigators and clinical site staff who are significant contributors to research must register in the Registration and Credential Repository (RCR) at <https://ctepcore.nci.nih.gov/rcr/>. The RCR is a self-service online person registration application with electronic signature and document submission capability.

RCR utilizes four person registration types that are applicable to this trial.

* Investigator (IVR): MD, DO, or international equivalent,
* Non Physician Investigator (NPIVR): advanced practice providers (*e*.*g*., NP or PA) or graduate level researchers (*e*.*g*., PhD),
* Associate Plus (AP): clinical site staff (*e*.*g*., RN or CRA) with data entry access to CTSU applications such as the Roster Update Management System (RUMS), OPEN, Rave, acting as a primary site contact, or with consenting privileges, and
* Associate (A): other clinical site staff involved in the conduct of NCI-sponsored trials.

RCR requires the following registration documents:

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Documentation Required | IVR | NPIVR | AP | A |
| FDA Form 1572 | ✔ | ✔ |  |  |
| Financial Disclosure Form | ✔ | ✔ | ✔ |  |
| NCI Biosketch (education, training, employment, license, and certification) | ✔ | ✔ | ✔ |  |
| GCP training | ✔ | ✔ | ✔ |  |
| Agent Shipment Form (if applicable) | ✔ |  |  |  |
| CV (optional) | ✔ | ✔ | ✔ |  |

IVRs and NPIVRs must list all clinical practice sites and Institutional Review Boards (IRBs) covering their practice sites in RCR to allow the following:

* Addition to a site roster,
* Selection as the treating, credit, or consenting person in OPEN,
* Ability to be named as the site-protocol Principal Investigator (PI) on the IRB approval, and
* Assignment of the Clinical Investigator (CI) task on the Delegation of Tasks Log (DTL).

In addition, all investigators acting as the Site-Protocol PI (Investigator listed on the IRB approval), consenting or treating investigator in OPEN, or as the Clinical Investigator (CI) on the DTL must be rostered at the enrolling site with a participating organization.

Refer to the [NCI RCR](https://ctep.cancer.gov/investigatorResources/default.htm) page on the [CTEP website](https://ctep.cancer.gov) for additional information. For questions, please contact the **RCRHelp Desk** by email at RCRHelpDesk@nih.gov.

## Site Registration

This study is supported by the NCI Cancer Trials Support Unit (CTSU).

**IRB Approval**

Sites participating through the NCI Central Institutional Review Board (NCI CIRB) must submit the Study Specific Worksheet (SSW) for Local Context to the CIRB using IRBManager to indicate their intent to open the study locally. The NCI CIRB’s approval of the SSW is automatically communicated to the CTSU Regulatory Office, but sites are required to contact the CTSU Regulatory Office at CTSURegPref@ctsu.coccg.org to establish site preferences for applying NCI CIRB approvals across their Signatory Network. Site preferences can be set at the network or protocol level. Questions about establishing site preferences can be addressed to the CTSU Regulatory Office by email (CTSURegPref@ctsu.coccg.org) or by calling 1-888-651-CTSU (2878).

In addition, the Site-Protocol PI (*i*.*e*., the investigator on the IRB/REB approval) must meet the following criteria for the site to be able to have an Approved status following processing of the IRB/REB approval record:

* Have an active CTEP status,
* Have an active status at the site(s) on the IRB/REB approval (*applies to US and Canadian sites only*) on at least one participating organization’s roster,
* If using NCI CIRB, be active on the NCI CIRB roster under the applicable CIRB Signatory Institution(s) record,
* Include the IRB number of the IRB providing approval in the Form FDA 1572 in the RCR profile,
* List all sites on the IRB/REB approval as Practice Sites in the Form FDA 1572 in the RCR profile; and
* Have the appropriate CTEP registration type for the protocol.

**Additional Requirements**

Additional site requirements to obtain an approved site registration status include:

* An active FederalWide Assurance (FWA) number,
* An active roster affiliation with the Lead Protocol Organization (LPO) or a Participating Organization (PO),
* An active roster affiliation with the NCI CIRB roster under at least one CIRB Signatory Institution (US sites only); and
* Compliance with all applicable protocol-specific requirements (PSRs).
	+ 1. Downloading Site Registration Documents

Download the site registration forms from the protocol-specific page located on the CTSU members’ website. Permission to view and download this protocol and its supporting documents is restricted to institutions and their associated investigators and staff on a participating roster. To view/download site registration forms:

* Log in to the CTSU members’ website ([https://www.ctsu.org](https://www.ctsu.org/)),
* Click on *Protocols* in the upper left of the screen
	+ Enter the protocol number in the search field at the top of the protocol tree, or
	+ Click on the By Lead Organization folder to expand, then select LAO-CA043, and protocol number 10699,
* Click on *Documents*, *Protocol Related Documents*, and use the *Document Type* filter and select *Site Registration* to download and complete the forms provided. (Note: For sites under the CIRB, IRB data will load automatically to the CTSU.)
	+ 1. Protocol Specific Requirements For 10699 Site Registration
		- None.
		1. Submitting Regulatory Documents

Submit required forms and documents to the CTSU Regulatory Office using the Regulatory Submission Portal on the CTSU members’ website.

To access the Regulatory Submission Portal, log on to the CTSU members’ website, go to the *Regulatory* section, and select *Regulatory Submission*.

Institutions with patients waiting that are unable to use the Regulatory Submission Portal should alert the CTSU Regulatory Office immediately by phone or email: 1-866-651-CTSU (2878), or CTSURegHelp@coccg.org to receive further instruction and support.

**Delegation of Tasks Log (DTL)**

Each site must complete a protocol-specific DTL using the DTL application which is accessible via the Delegation Log link on the CTSU members’ website or directly at <https://dtl.ctsu.org>. The Clinical Investigator (CI) is required to review and electronically sign the DTL prior to the site receiving an approved site registration status and enrolling patients to the study. To maintain an approved site registration status the CI must re-sign the DTL at least annually and when a new version of the DTL is released; and activate new task assignments requiring CI sign-off. Any individual at the enrolling site on a participating roster may initiate the site DTL. Once the DTL is submitted for CI approval, only the designated DTL Administrators or the CI may update the DTL. Instructions on completing the DTL are available in the Help Topics button in the DTL application and describes DTL task assignments, CI signature, and CTEP registration requirements, as well as include a Master Task List.

The DTL for this study has training requirements as follows:

1. All staff assigned the Rave CRA task on the DTL for this study must complete the *Theradex Specimen Tracking System (STS) Training* course. The training is a one-time only requirement per individual. If an individual has previously completed the training for another ETCTN study with the same course requirement, the training does not need to be completed again. However, new versions of the Specimen Tracking System may require new training.

A task-related training requirement is satisfied by completing the course in the Compliance, Learning, and SOP Solutions (CLASS) application at <https://classlms.org>; until that takes place, the task assignment will remain in a Pending status.

* When a Rave CRA is assigned the task on an ETCTN DTL, the system will check to see if they have completed the appropriate course, and if not, they will be automatically enrolled in the course. They will receive an enrollment email from CLASSHelpDesk@westat.com that will include instructions on how to access the course.

When an assignee completes the necessary course, their completion will be automatically communicated from CLASS to the DTL application and the task status will go to Active or Awaiting CI Approval, depending on the situation (please allow up to four hours for this to happen). The DTL cannot be submitted for CI sign-off until the minimum number of persons are assigned to the task(s) and have met all task requirements including any training requirements.

* + 1. Checking Site Registration Status

Site’s registration status may be verified on the CTSU members’ website.

* Click on *Regulatory* at the top of the screen,
* Click on *Site Registration*, and
* Enter the site’s 5-character CTEP Institution Code and click on Go.
	+ Additional filters are available to sort by Protocol, Registration Status, Protocol Status, and/or IRB Type.

Note: The status shown only reflects institutional compliance with site registration requirements as outlined within the protocol. It does not reflect compliance with protocol requirements for individuals participating on the protocol or the enrolling investigator’s status with NCI or their affiliated networks.

## Patient Registration

* + 1. OPEN / IWRS

The Oncology Patient Enrollment Network (OPEN) is a web-based registration system available on a 24/7 basis. OPEN is integrated with CTSU regulatory and roster data and with the LPOs registration/randomization systems or the Theradex Interactive Web Response System (IWRS) for retrieval of patient registration/randomization assignment. OPEN or IWRS will populate the patient enrollment data in NCI’s clinical data management system, Medidata Rave.

Requirements for OPEN access:

* Active CTEP registration with the credentials necessary to access secure NCI/CTSU IT systems.
* To perform enrollments or request slot reservations: Must be on an LPO roster, ETCTN corresponding roster, or PO roster with the role of Registrar. Registrars must hold a minimum of an Associate Plus (AP) registration type.
* If a DTL is required for the study, the registrar must hold the OPEN Registrar task on the DTL for the site.
* Have an approved site registration for the protocol prior to patient enrollment.

To assign an Investigator (IVR) or Non-Physician Investigator (NPIVR) as the treating, crediting, consenting, or receiving investigator for a patient transfer in OPEN, the IVR or NPIVR must list the Institutional Review Boards (IRB) number used on the site’s IRB approval on their Form Food and Drug Administration (FDA) 1572 in Registration and Credential Repository (RCR). If a DTL is required for the study, the IVR or NPIVR must be assigned the appropriate OPEN-related tasks on the DTL.

Prior to accessing OPEN, site staff should verify the following:

* Patient has met all eligibility criteria within the protocol stated timeframes, and
* All patients have signed an appropriate consent form and Health Insurance Portability and Accountability Act (HIPAA) authorization form (if applicable).

Note:  The OPEN system will provide the site with a printable confirmation of registration and treatment information. IWRS system also sends an email confirmation of the registration. You may print this confirmation for your records.

Access OPEN at <https://open.ctsu.org> or from the OPEN link on the CTSU members’ website. Further instructional information is in the OPEN section of the CTSU website at <https://www.ctsu.org> or <https://open.ctsu.org>. For any additional questions, contact the CTSU Help Desk at 1-888-823-5923 or ctsucontact@westat.com.

Patient enrollment for this study will be facilitated using the Slot Reservation System in conjunction with patient enrollment in OPEN. Prior to discussing protocol entry with the patient, all site staff must use the CTSU OPEN Slot Reservation System or the IWRS Slot Reservation System to ensure that a slot on the protocol is available to the patient. Once a slot reservation confirmation is obtained, site staff may then proceed to enroll the patient to this study.

* + 1. Special Instructions for Patient Enrollment
1. Site staff with the appropriate roles will reserve slots using IWRS (<https://open.ctsu.org/>).
2. City of Hope Cancer Center will receive notification via the IWRS when a slot has been reserved. An e-mail will be sent from the City of Hope Cancer Center to the site requesting further information such as: patient initials, tumor type, and potential start date. The spot will show as ‘pending approval’ in the system until the site sends a REGISTRATION FORM/ELIGIBILITY CHECKLIST (posted in the CTSU members’ website under NCI# 10699) accompanied with documents supporting eligibility (signed consent, baseline labs, pathology reports, MRI reports, and latest clinic note) to the City of Hope Cancer Center at ccc@coh.org for review and confirmation of eligibility.
3. Once the registration has been reviewed, the City of Hope Cancer Center will either approve or disapprove the request depending on confirmation of patient eligibility. If approved, the City of Hope Cancer Center will update the spot to ‘reserved’ in IWRS.
4. The site can now enroll the patient into the study in OPEN.
	* 1. OPEN/IWRS Questions?

Further instructional information on OPEN is provided on the OPEN link of the CTSU website at <https://www.ctsu.org> or at <https://open.ctsu.org>. For any additional questions contact the CTSU Help Desk at 1-888-823-5923 or ctsucontact@westat.com.

Theradex has developed a Slot Reservations and Cohort Management User Guide, which is available on the Theradex website: <http://www.theradex.com/clinicalTechnologies/?National-Cancer-Institute-NCI-11>. This link to the Theradex website is also on the CTSU website OPEN tab. For questions about the use of IWRS for slot reservations, contact the Theradex Helpdesk at 855-828-6113 or Theradex main number 609-799-7580; CTMSSupport@theradex.com.

## General Guidelines

Following registration, patients should begin protocol treatment within 7 days. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a patient does not receive protocol therapy following registration, the patient’s registration on the study may be canceled. The Study Coordinator should be notified of cancellations as soon as possible.

# BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

## Summary Table for Specimen Collection

| **Time Point** | **Specimen** | **Send Specimens To:** |
| --- | --- | --- |
| **Archival1, 2** |
| All Patients on the treatment portion of the study | * Formalin-fixed paraffin-embedded (FFPE) tumor tissue block (preferred) (optional)3, 4

If a block is not available, then submit:* 1 H&E stained slide (3-5 µm)
* 30-50 unstained, uncharged, air-dried slides (10 µm). If not feasible, then a minimum of 20 unstained air-dried uncharged slides (10 µm) should be submitted with a minimum tumor content of 30-40%.5
 | EET BiobankNote: for patients at City of Hope on the window-of- opportunity PK sub-study, tissue should not be submitted to the EET Biobank unless they continue onto the treatment portion of the study. |
| **At least 24 h after surgery during window-of-opportunity PK sub-study** |
| Only for select patients at City of Hope on the window-of-opportunity PK sub-study:* Pre-dose & 0.5, 1, 1.5, 2, 3, 4, 6, and 8 h post-dose
 | * 3-5 mL blood in a purple-top EDTA tube, processed to plasma, and frozen per time point (mandatory)
 | Analytical Pharmacology Core, City of Hope |
| Only for select patients at City of Hope on the window-of-opportunity PK sub-study:* Pre-dose & every hour up to 24 h post-dose
 | * At least 15 mcL CSF in a microvial (Ref # P00001, M Dialysis, Stockholm, Sweden) will be continuously collected at each time point and frozen (mandatory)
 | Analytical Pharmacology Core, City of Hope |
| **Baseline** |
| All Patients on the treatment portion of the study* Pre-dose
 | * 10 mL blood in K2 EDTA tube, shipped ambient (mandatory, if tissue submitted for WES)
 | EET BiobankNote: for patients at City of Hope on the window-of- opportunity PK sub-study, blood should not be submitted to the EET Biobank unless they continue onto the treatment portion of the study. |
| **Week 1 Day 1** |
| All Patients on the treatment portion of the study* 1-3 h post-dose
 | * 3-5 mL blood in a purple-top EDTA tube, processed to plasma, and frozen (mandatory)
 | Analytical Pharmacology Core, City of Hope |
| **Week 1 Day 2 (Alternative radiation treatment days acceptable)** |
| All Patients on the treatment portion of the study* Pre-dose & 0.5, 1, 1.5, 2, 3, 4, 6, and 8 h post-dose
 | * 3-5 mL blood in a purple-top EDTA tube, processed to plasma, and frozen per time point (mandatory)
 | Analytical Pharmacology Core, City of Hope |
| 1Archival tumor tissue from initial diagnosis or recurrence are allowed. No re-biopsies planned/allowed.2For City of Hope patients scheduled for surgery, FFPE surgical tissue may be submitted instead of archival tissue.3For archival biopsy tissue, **a copy of the anatomic pathology report corresponding to the tissue collection procedure must be sent with the tissue and uploaded to Rave**. If submitting slides, then slides must be processed in order, and numbered sequentially (*e.g*., H&E stained slide is created first and labeled 1, unstained slides are then created and numbered 2 – 51).4For FFPE surgical tissue, **upload the Corresponding Pathology Report from the surgery to Rave and send a copy to the EET Biobank** with the tissue**.**  Please hold submission of the FFPE surgical tissue to the EET Biobank until the Corresponding Pathology Report is available.**5Submission of specimens with <30% tumor content may not provide sufficient material for analysis.** |

## Summary Table(s) for Interventional Radiologist for Research Biopsies

Not applicable.

## Specimen Procurement Kits and Scheduling

* + 1. Specimen Procurement Kits

Kits for the collection and shipment of select specimens to the EET Biobank can be ordered online via the Kit Management system: (<https://kits.bpc-apps.nchri.org>).

Users at the clinical sites will need to set up an account in the Kit Management system and select a specific clinical trial protocol to request a kit. Please note that protocol may include more than one type of kit. Each user may order two kits per kit type per day (daily max = 6 kits). Kits are shipped ground, so please allow 5-7 days for receipt. A complete list of kit contents for each kit type is located on the Kit Management system website.

**Note:** Kits or supplies are only provided for specimens shipped to the EET Biobank. Institutional supplies must be used for all other specimen collection and processing.

* + 1. Scheduling of Specimen Collections
			1. Scheduling of Specimen Collection to the EET Biobank

Please adhere to the following guidelines when scheduling procedures to collect tissue:

* Tissue submitted as FFPE (blocks or slides) can be collected on any day but must be shipped to the EET Biobank on Monday through Thursday.
* Fresh blood specimens may be collected and shipped Monday through Friday.
	+ - 1. Scheduling of Specimen Collection to Analytical Pharmacology Core, City of Hope
* Plasma and CSF specimens are to be shipped on either Monday, Tuesday, or Wednesday.

## Specimen Tracking System Instructions

This Study will use the ETCTN Specimen Tracking System (STS).

* All biospecimens collected for this trial must be submitted using the ETCTN STS unless otherwise noted.
* The system is accessed through Rave user roles: “Rave CRA” and “Rave CRA (Labadmin)” for data entry at the treating institutions. The “Biorepository” role is assigned to users receiving the specimens for processing and storage at reference labs and the NCI Early-Phase and Experimental Clinical Trials Biospecimen Bank (EET Biobank, formerly known as the ETCTN Biorepository).

**Important:** **Failure to complete required fields in STS may result in a delay in sample processing.** Any case reimbursements associated with sample submissions will not be credited if samples requiring STS submission are not logged into STS.

Detailed instructions on use of the STS can be found below.

* + 1. Specimen Tracking System Overview and Enrollment Instructions

For the ETCTN STS, the following information will be requested:

* Protocol Number
	+ - Investigator Identification
	+ Institution and affiliate name
	+ Investigator’s name
* Eligibility Verification: Patients must meet all the eligibility requirements listed in Section 3.
* Additional Requirements:
* Patients must provide a signed and dated, written informed consent form.

Upon enrolling a patient, IWRS will communicate with OPEN, assigning two separate and unique identification numbers to the patient, a Universal patient ID (UPID) and a Treatment patient ID. The UPID is associated with the patient and used each and every time the patient engages with the portion of this or any other protocol that uses the ETCTN Specimen Tracking System. The UPID contains no information or link to the treatment protocol. IWRS will maintain an association between the UPID for ETCTN biobanking and molecular characterization and any treatment protocols the patient participates in, thereby allowing analysis of the molecular characterization results with the clinical data.

Immediately following enrollment, the institutional anatomical pathology report for the diagnosis under which the patient is being enrolled must be uploaded into Rave. The report must include the surgical pathology ID (SPID), collection date, block number, and the IWRS-assigned UPID and patient study ID for this trial. For newly acquired biopsies without a corresponding pathology report, the radiology and operative report(s) must also be uploaded into Rave, when available. **Important: Remove any personally identifying information, including, but not limited to, the patient’s name, date of birth, initials, medical record number, and patient contact information from the institutional pathology report prior to submission.**

Additionally, please note that the STS software creates pop-up windows when reports are generated, so you will need to enable pop-ups within your web browser while using the software.

For questions regarding the Specimen Tracking System, please contact STS Support at STS.Support@theradex.com.

The Shipping List report **must** be included with all sample submissions.

* + 1. Specimen Labeling
			1. Blood and CSF Specimen Labels

Include the following on blood and CSF specimens (including whole blood and frozen, processed blood products – like plasma):

* Patient Study ID
* Universal Patient ID (UPID)
* Specimen ID (automatically generated by Rave)
* Time point
* Specimen type (*e.g.*, blood, plasma, CSF)
* Collection date (all specimens) and time [for PK plasma and CSF specimens only(to be added by hand)]. Note: the time should be recorded using a 24 hour clock (HH:MM).
	+ - 1. Tissue Specimen Labels

Include the following on all tissue specimens (*e.g.*, FFPE block, slides, or frozen tissue) or containers (*e.g.*, formalin jar):

* Patient Study ID
* Universal Patient ID (UPID)
* Specimen ID (automatically generated by Rave)
* Time point
* Specimen type (*e.g.*, formalin-fixed paraffin-embedded [FFPE] Block, Formalin Fixed Tissue, Fresh Tissue in Media, *etc.*)
* Tissue type (P for primary, M for metastatic or N for normal)
* Surgical pathology ID (SPID) number (when applicable)
* Block number from the corresponding pathology report (FFPE tissue, when applicable)
* Collection date (to be added by hand)
* Slide section number (only if archival tissue is submitted as slides) (to be added by hand)
	+ - 1. Example of Specimen Label Generated by STS

STS includes a label printing facility, accessed via the Print Label CRF in the All Specimens folder. A generated PDF is emailed to the user as a result of saving that form.

The following image is an example of a tissue specimen label printed on a label that is 0.5” high and 1.28” wide.



The QR code in the above example is for the Specimen ID shown on the second line.

Labels may be printed on a special purpose label printer, one label at a time, or on a standard laser printer, multiple labels per page. Theradex recommends the use of these low temperature waterproof labels for standard laser printers: <https://www.labtag.com/shop/product/cryo-laser-labels-1-28-x-0-5-cl-23-colors-available/>

The last line item on the label includes the following data points joined together:

1. Tissue only: Primary (P), Metastatic (M), Normal (N) tissue indicated at the beginning of the specimen ID; this field is blank if not relevant (*e*.*g*., for blood)
2. Block ID or blank if not relevant
3. SPID (Surgical Pathology ID) or blank if none
4. An optional alpha-numeric code that is protocol specific and is only included if the protocol requires an additional special code classification

**Space is provided at the bottom of the label for the handwritten date and optional time.** The last line on the example label is for the handwritten date and optional time.

* + 1. Overview of Process at Treating Site
			1. OPEN Registration

All registrations will be performed using the Oncology Patient Enrollment Network (OPEN) system. OPEN communicates automatically with the Interactive Web Response System (IWRS) which handles identifier assignments, any study randomization, and any prescribed slot assignments. If specimen analysis is required to determine eligibility, the protocol will be setup with multi-step registration.

Registration without eligibility specimen analysis:

1. Site enters registration data into OPEN during one or more steps.
2. IWRS receives data from OPEN, generates the Patient Study ID and the Universal Patient ID, both of which are sent back to OPEN.
3. IWRS sends all applicable registration data directly to Rave at the end of the final registration step.

Any data entry errors made during enrollment should be corrected in Rave.

* + - 1. Rave Specimen Tracking Process Steps

**Step 0**: Log into Rave via your CTEP-IAM account, then navigate to the appropriate participant.

**Step 1**: Complete the **Histology and Disease** form (but do not upload reports until a specimen label can be applied to them) and the Baseline forms regarding **Prior Therapies**. Enter the initial clinical specimen data:

* **Specimen Tracking Enrollment** CRF: Enter Time Point, Specimen Category, Specimen Type, Block number, Tissue type, Surgical Path ID, and number of labels needed (include extra labels to apply to reports to be uploaded). CRF generates unique Specimen ID.

**Step 2**: Print labels using the **Print Labels** CRF located in the All Specimens folder, then collect specimen.

* Label specimen containers and write collection date (all specimens) and time (for PK plasma and CSF specimens only)on each label. After collection, store labeled specimensas described in Section 5.5.
* Apply an extra specimen label to *each* report before scanning. Return to the **Histology and Disease** form to upload any initial Pathology, Radiology, Molecular Reports (up to 4), and Surgical (or Operative) reports. Return to **Specimen Tracking Enrollment** CRF to upload any molecular report (one per specimen) and/or specimen specific pathology or related report (one per specimen) and/or the Tissue Biopsy Verification form (when applicable). Uploaded reports should have protected health information (PHI) data, like name, date of birth, mailing address, medical record number or social security number (SSN), redacted**. Do not redact SPID, block number, diagnosis or relevant dates (such as collection date), and include the UPID and patient study ID on each document** (either by adding a label or hand writing).

**Step 3**: Complete specimen data entry.

* **Specimen Transmittal** Form: Enter collection date and time and other required specimen details.

**Step 4**: When ready to ship, enter shipment information.

* **Shipping Status** CRF: Enter tracking number, your contact information, recipient, number of sample containers and ship date once for the first specimen in a shipment.
* **Copy Shipping** CRF: In the specimen folders for additional specimens (if any) that will be shipped with the initial specimen, please use the **Copy Shipping** form to derive common data into additional **Shipping Status** forms. A few unique fields will still need to be entered in **Shipping Status.**

**Step 5**: Print shipping list report and prepare to ship.

* Shipping List report is available at the site level.
* Print two copies of the shipping list, one to provide in the box, the other for your own records.
* Print pathology or other required reports to include in the box. Be sure the printed copy includes the specimen label.

**Step 6**: Send email notification.

* For only one of the specimens in the shipment, click “Send Email Alert” checkbox on the **Shipping Status** CRFto email recipient.

**Step 7:** Ship the specimen(s).

**Step 8**: Monitor the Receiving Status form located in each specimen folder for acknowledgment of receipt and adequacy.

## Specimen Collection

* + 1. Archival or Formalin-Fixed Paraffin-Embedded (FFPE) Tumor Specimen (EET Biobank)

If previously-collected FFPE tissue will be submitted, then the following criteria must be met:

* Tissue from initial diagnosis or recurrence is acceptable. Please submit the most recent archival tissue that is available. While not required, it is preferable if the tissue submitted was collected < 6 months from registration for optimum assay results.
* FFPE tumor tissue block(s) must be submitted. The optimal block is at least 70% tumor. Specimen size requirement is as follows:
	+ Surface area: 25 mm2 is optimal. Minimum is 5 mm2.
	+ Volume: 1 mm3 optimal. Minimum volume is 0.2 mm3, however the success of DNA extraction decreases at suboptimal tissue volume.

If an existing block cannot be submitted, the following are requested, if available:

* One (1) H&E slide (3-5 µm)
* Thirty to fifty (30 – 50) 10 µm unstained air-dried uncharged slides (preferred). If not feasible, then a minimum of twenty (20) 10 µm unstained air-dried uncharged slides should be submitted with a minimum tumor content of at least 30%. **Submission of specimens with <30% tumor content may not provide sufficient material for analysis**

Process and number slides sequentially (e.g., H&E stained slide should be created first and labeled with “1,” and additional unstained slides should be processed next and be labeled 2 – 51).

See Section 5.4.2 for labeling instructions.

* + 1. Blood Collection
			1. Collection of Blood in K2 EDTA Tubes for Shipping Whole Blood (EET Biobank)
1. Label EDTA tubes according to the instructions in Section 5.4.2.
2. Collect 10 mL blood in EDTA tube(s) and gently invert tube to mix.
3. Ship on day of collection (whenever possible) according to instructions in Section 5.6.
4. If blood cannot be shipped on the day of collection (e.g., a late scheduled collection), then refrigerate until shipment.
	* + 1. Collection of Blood in Purple-Top EDTA Tubes for Plasma Processing (Analytical Pharmacology Core, City of Hope)
5. An in-dwelling heparin catheter will be placed for serial specimen collection.
6. Label EDTA tube(s) according to the instructions in Section 5.4.2.
7. At each sampling time, draw 1 mL of blood and discard. This assures the solution used to maintain the catheter patency does not dilute the sample.
	1. The sampling times are as follows: Week 1, Day 1: 1-3 hours post-dose; Week 1, Day 2 (alternative days during radiation treatment are acceptable): pre-dose, 0.5-, 1-, 1.5-, 2-, 3-, 4-, 6-, and 8-hours post-dose ([Appendix F](#_APPENDIX_F_PK) and [G](#_APPENDIX_G_PK)).
8. For each plasma PK sample, collect 3-5 mL anticoagulated whole blood in a purple top EDTA tube (*e.g.,* BD vacutainer 367861 plastic 13 x 75 4 mL tube).
9. Invert the vacutainer tubes several times to mix blood with EDTA anticoagulant and immediately place on ice.
10. Processing should begin within 30 minutes of collection.
11. Process plasma by centrifuging for 10 minutes at 1,000 × g in a refrigerated tabletop centrifuge.
12. Using a clean transfer pipette, transfer plasma into an appropriately labeled cryovial (using the label printed from the ETCTN Specimen Tracking System or following the instructions in Section 5.4.1). Avoid picking up the blood cells when aliquoting by keeping the pipet above the cell layers and leaving a small amount of plasma in the tube. Tightly secure the cap of the vials before storage. Aliquoting and freezing of plasma specimens should be completed within 1 hour of centrifugation.
13. Store plasma cryovials upright in a specimen box or rack in an -70°C to -90°C or colder freezer prior to delivering to laboratory. Do not allow specimens to thaw after freezing.
14. Ship according to instructions in Section 5.7.
	* + 1. Collection of CSF in Microvial (Analytical Pharmacology Core, City of Hope) (Applicable only for select City of Hope patients participating in window-of-opportunity microdialysis sub-study)
15. After tumor resection, one microdialysis catheter will be placed in tumor and/or peritumoral tissue, depending on the extent of the resection performed and per neurosurgeon’s judgment as to what is feasible.
	1. The 70 Brain Microdialysis Catheter (membrane length 10mm and shaft length 100mm (Ref # P000050, M Dialysis, Stockholm, Sweden) will be flushed with sterile artificial CSF prior to placement in brain tissue and tunneled subcutaneously away from the craniotomy or burr hole.
	2. Wound will be irrigated and closed in standard fashion, anchoring the catheter to the scalp with suture and sterile dressings.
16. A computed tomography (CT) scan without contrast will be obtained as soon as possible after placement of the catheter(s) to determine correct positioning.
	1. In those cases where there is significant tumor bed hemorrhage that would preclude probe access to brain or tumor tissue, or when there is poor positioning of the catheter, it will be removed.
17. Once the CT scan confirms that the microdialysis catheter has been placed appropriately, the inlet tubing of the catheter(s) will be connected to a 2.5 mL syringe (106 Pump Syringe, Ref # 8010191, M Dialysis, Stockholm, Sweden) filled with sterile artificial CSF (Perfusion Fluid CNS: NaCl 147 mmol/L, KCl 2.7 mmol/L, CaCl2 1.2 mmol/L, MgCl2 0.85 mmol/L; Ref # P000151, M Dialysis, Stockholm, Sweden). A new 2.5mL syringe will be replaced every 24 hours.
	1. The syringe will be placed in a portable syringe pump (107 MD Pump, Ref # P000127, M Dialysis, Stockholm, Sweden).
	2. The flow control on the pump will be set at 0.5 µL/min and the pump will be turned on.
	3. A plastic microvial (Ref #P000001, M Dialysis, Stockholm, Sweden) will be placed in the vial holder at the end of the outlet tubing to collect dialysate.
	4. This microvial can hold up to 200 µL of fluid. It will need to be replaced with a new one every 3 hours until the dose of triapine is given on Post-op Day 1 (or later).
18. After surgery, the patient will be transferred to the Intensive Care Unit (ICU) for monitoring of the microdialysis pump and collection of the dialysate samples as outlined below.
	1. The patient will either remain in the ICU or, when medically stable, can be transferred to a regular floor bed as long as nursing can be provided to the patient until all of the dialysate samples have been collected.
19. Prior to the first dose of triapine, place a new microvial in the holder of the outlet tubing.
20. Microvials will continuously collect dialysate and microvials will be collected at specified time points: before dose of triapine and then every hour following the dose up to 24 hours after the dose ([Appendix H](#_APPENDIX_H_Microdialysis)).
	1. A new microvial will be replaced at each time point.
	2. Sequentially label the microvial(s) at the time of collection.
	3. It is important not to flush the catheter (unless it becomes clogged, as indicated by dialysate not exiting the outlet tubing) after the dose of triapine is administered. If the pump needs to be stopped, (*e.g.,* to place a new syringe of artificial CSF in it), then stop the pump by taking the batteries out, not by opening the lid. Whenever the lid is opened and then closed, the pump automatically goes into a flush cycle.
	4. Microvials containing the dialysate samples will be placed on dry ice until they can be moved to an ultralow temperature freezer, where they will be stored until shipped according to instructions in Section 5.7. Label the storage box according to instructions in Section 5.4.2.
	5. During the period of dialysate collection, a patient may be mobile within the confines of the collection system. For example, s/he may move from bed to chair or commode.
21. After all the dialysate samples have been collected, the microdialysis catheter will be removed percutaneously at the bedside.
	1. Xylocaine for local anesthesia will be used as needed.
	2. The entry site of the catheter will be closed with a suture or steri-strips as necessary and a clean dressing applied.
	3. If the patient has post-operative complications, and it is not possible to proceed with microdialysis within 72 hours of placing the catheter, then the catheter will be removed.
22. The catheter will be placed in zip-lock plastic bags and stored with the study samples. The patient will be discharged from the hospital when medically ready.

## Shipping Specimens from Clinical Site to the EET Biobank

* + 1. General Shipping Information
			1. Required Forms for Specimen Submissions

| **Specimen**  | **Required Forms** |
| --- | --- |
| Archival Tissue | 1. Shipping List
2. Anatomic Pathology Report corresponding to the tissue collection procedure
 |
| Surgical Tissue*Only applicable to City of Hope sub-study patients who do not have archival tissue available.* | 1. Shipping List
2. Corresponding Pathology Report from the surgery1

1For FFPE tissue, please hold submission of the tissue to the EET Biobank until the Corresponding Pathology Report is available. |
| Other (blood, CSF) | 1. Shipping List |

**Each document submitted with the specimen must be labeled with a label printed from the STS, or the Universal ID and Patient Study ID.**

Minimum required personally identifiable information:

* Remove patient identifiers such as name, date of birth, medical record number, social security number, and insurance information from the pathology or other clinical reports.
* Do not remove the date of procedure, surgical pathology ID (SPID) number, block number, and diagnosis.
	+ 1. Specimen Shipping Instructions

When kits are provided, the shipping container sent with kit contents should be used to ship specimens to the EET Biobank. In winter months, please include extra insulation, such as bubble wrap, inside the shipping container with ambient specimens.

Archival (FFPE) tissue may be shipped on Monday through Thursday.

Fresh blood may be shipped on Monday through Friday. Please select “Saturday Delivery” when shipping fresh blood on a Friday.

* + - 1. Shipping of FFPE Blocks and Glass Slides
1. Before packaging blocks or slides, verify that each specimen is labeled according to Section 5.4.2.2.
2. Blocks should be placed in a hard-sided container, preferably a special block holder, to protect the specimen. Glass slides are to be placed in plastic slide holders. Place tissue paper on top of the separated slides prior to closing the slide holder to reduce slide movement during shipment.
3. Place the blocks or slides in a reinforced cardboard shipping box with appropriate packaging filler to minimize movement of specimens within the shipping box.
4. Include a copy of the forms listed above and a shipping manifest from the Specimen Tracking System with each shipment.
5. Please include a cold pack when shipping on hot days and extra insulation on cold days.
6. Ship specimens to the address listed below. FedEx Priority Overnight is strongly recommended to prevent delays in package receipt.
	* + 1. Shipping Ambient Blood Using Supplies Provided by the Institution
7. Before packaging specimens, verify that the collection tube is labeled according to instructions in section 5.4.2.1.
8. Place the blood collection tube into a zip-lock bag.
9. Place zip-lock bag into a biohazard envelope with absorbent material. Expel as much air as possible and seal the envelope securely.
10. Place the biohazard envelope into a Tyvek envelope. Expel as much air as possible and seal securely.
11. Place the specimen(s) and a copy of the shipping manifest into a sturdy shipping container. In winter months please use an insulated container and include extra insulation, such as bubble wrap, inside the shipping container to prevent specimens from freezing.
12. Close the container and tape shut.
13. Attach a shipping label to the top of the shipping container.
14. Attach an Exempt Human Specimen sticker to the side of the container.
15. Ship specimens via overnight courier to the address below. FedEx Priority Overnight is strongly recommended to prevent delays in package receipt.
	* 1. Shipping Address

Ship to the address below. Ship formalin-fixed and fresh blood specimens the same day of specimen collection. Do not ship specimens the day before a holiday.

EET Biobank

2200 International Street

Columbus, Ohio 43228

PH: (614) 722-2865

FAX: (614) 722-2897

E-mail: BPCBank@nationwidechildrens.org

**FedEx Priority Overnight** service is very strongly preferred.

**NOTE:** The EET Biobank FedEx Account will not be provided to submitting institutions. There is no central Courier account for this study. Sites are responsible for the cost of shipments to the EET Biobank.

* + 1. Contact Information for Assistance

For all queries, please use the contact information below:

EET Biobank

PH: (614) 722-2865

E-mail: BPCBank@nationwidechildrens.org

## Shipping of Specimens from Clinical Site to Other Laboratories

* + 1. Shipping of Specimens to Analytical Pharmacology Core, City of Hope
			1. Specimen Shipping Instructions

All samples should be shipped via overnight express courier in insulated contains with enough dry ice to maintain the samples in a frozen state. Samples should be stored in cardboard boxes (5 1/8’ x 5 1/8’ x 2’, L x W x H). Samples should be organized by patient and time point in the box. Samples will not be placed in plastic bags. A copy of each pharmacokinetic sample collection forms for the respective patients should be included with each shipment. To prevent problems with illegible writing on tubes, consider numbering them and numbering the samples on the sample sheet.

* + - 1. Shipping Address

Analytical Pharmacology Core

City of Hope

Room 1042 Shapiro Building

1500 E. Duarte Road

Duarte, CA 91010

* + - 1. Contact Information for Assistance

PK Director: Tim Synold, Pharm.D.

Lab phone: 626-218-1110

Lab fax: 626-471-9376

PK lab email: tsynold@coh.org

## Biomarker Plan

**List of Biomarker Assays in Order of Priority**

***Note for participating sites: Please see Section 5.1 for details on specimens to collect. The specimens tested are not always the same specimens that are submitted by the site, as processing of blood and tissue will occur at the Biobank prior to testing.***

| **Priority** | **Biomarker Name** | **Assay and****CLIA: Y/N** | **Use in the Trial and Purpose** | **Specimens Tested** | **Collection Time Points** | **Mandatory or Optional** | **Assay Laboratory, Lab PI, and Lab PI Email** |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Tissue-based**  |
| N/A | IDH1/2 | NGSCLIA: Y | IntegratedTo correlate IDH1/2 status with tumor response and clinical outcomes | N/A | All patients:Pre-enrollmentWill be collected from pre-existing patient records from diagnosis | M  | Local Testing |
| N/A | MGMT Methylation Status | Methylation AssayCLIA: Y | IntegratedTo correlate MGMT-methylation status to tumor response and clinical outcomes | N/A | All patients:Pre-enrollmentWill be collected from pre-existing patient records from diagnosis | M  | Local Testing |
| 1 | Whole exome sequencing (WES) | NGSCLIA: N | IntegratedTo correlate genetic mutations with tumor response and clinical outcomes (e.g. TP53, p16, KRAS and PI3K/mTOR/ AKT)To retrospectively confirm IDH1/2 mutational status | DNA from FFPE tumor tissue | All patients on the treatment portion of the study:Archival1,2 | O | MoCha, Frederick National Laboratory for Cancer Research (FNLCR)Chris Karlovichchris.karlovich@nih.gov |
| 2 | RNAseq | NGSCLIA: N | IntegratedTo correlate RRM2 expression and other molecular signatures (e.g., radiosensitivity index, Buffa hypoxia score, inflammatory transcripts) with tumor response and clinical outcomes | RNA from FFPE tumor tissue | All patients on the treatment portion of the study:Archival1,2 | O | MoCha, Frederick National Laboratory for Cancer Research (FNLCR)Chris Karlovichchris.karlovich@nih.gov |
| **Blood-based** |
| 1 | Triapine PK | LC-MS/MS assayCLIA: N | IntegratedTo evaluate pharmacokinetics of intervention drug  | Plasma from blood in EDTA Tube | All patients on the treatment portion of the study:Week 1 Day 1: 1-3 h post doseWeek 1 Day 2 (alternative treatment days acceptable): pre, 0.5, 1, 1.5, 2, 3, 4, 6, 8 h post doseOnly for select patients at City of Hope on the window-of-opportunity PK sub-study:At least 24 h after surgery: pre, 0.5, 1, 1.5, 2, 3, 4, 6, 8 h post dose | MMM | Analytical Pharmacology Core, City of HopeTim SynoldTSynold@coh.org |
| 2 | Whole exome sequencing (WES) | NGSCLIA: N | IntegratedGermline control for WES | Germline DNA from blood in K2 EDTA tube | All patients on the treatment portion of the study:Baseline | M, if tissue submitted for WES | MoCha, Frederick National Laboratory for Cancer Research (FNLCR)Chris Karlovichchris.karlovich@nih.gov |
| **CSF-Based** |
| 1 | Triapine PK | LC-MS/MS assayCLIA: N | IntegratedTo evaluate pharmacokinetics of intervention drug | Peri-tumoral dialysate | Only for select patients at City of Hope on the window-of-opportunity PK sub-study:At least 24 h after surgery: pre-dose and then every hour up to 24 h post dose | M | Analytical Pharmacology Core, City of HopeTim SynoldTSynold@coh.org |

1Archival tumor from initial diagnosis or recurrence are allowed. No re-biopsies planned/allowed.

2For patients scheduled for surgery, surgical tissue may be used instead of archival.

## Integrated Correlative Studies

* + 1. IDH1/2
			1. Specimen(s) Receipt and Processing at Local Labs

Local labs should process these samples per institutional SOPs as part of standard of care.

* + - 1. Site(s) Performing Correlative Study

This study will be conducted at local CLIA certified labs.

* + - 1. Contact Information for Notification of Specimen Shipment

Not applicable.

* + 1. MGMT Methylation Status
			1. Specimen(s) Receipt and Processing at Local Labs

Local labs should process these samples per institutional SOPs as part of standard of care.

* + - 1. Site(s) Performing Correlative Study

This study will be conducted at local CLIA certified labs.

* + - 1. Contact Information for Notification of Specimen Shipment

Not applicable.

* + 1. Whole Exome Sequencing
			1. Specimen(s) Receipt and Processing at the EET Biobank

The EET Biobank will receive FFPE tumor tissue and blood from participating sites.

FFPE tissue will be received as an FFPE tissue block or an H&E-stained and unstained slides. FFPE tissue blocks and stained slides will be stored at room temperature, and unstained slides will be vacuum sealed and banked in refrigerated storage until processing.

H&E stained slides will undergo a pathology QA review to assess tumor content and annotate for macrodissection, when needed. Following macrodissection, tumor tissue from unstained slides will be scraped for co-extraction of DNA and RNA. The nucleic acids will be analyzed to determine concentration and quality prior to storage in a -80°C freezer. The remaining FFPE block and H&E stained slides will be stored at room temperature. Aliquots of DNA will be shipped for analysis.

At baseline, germline DNA will be extracted from whole blood collected in EDTA tubes. DNA will be quantitated and then stored in a -80°C freezer. An aliquot of germline DNA will be shipped for analysis.

* + - 1. Site(s) Performing Correlative Study

The assay will be performed at MoCha, Frederick National Laboratory for Cancer Research (FNLCR) under the supervision of Chris Karlovich, PhD.

* + - 1. Shipment of specimens from the EET Biobank to Site Performing Correlative Study

Specimens will be shipped from the EET Biobank to:

MoCha Lab, Frederick National Laboratory for Cancer Research (FNLCR)

1050 Boyles St.

Bldg. 459, Rm. 125

Frederick, MD 21702

Attn: Alyssa Chapman or Ruth Thornton

* + - 1. Contact Information for Notification of Specimen Shipment

Thomas Forbes, mochasamplereceiving@nih.gov

* + 1. RNAseq
			1. Specimen(s) Receipt and Processing at the EET Biobank

FFPE tissue will be received as an FFPE tissue block or an H&E-stained and unstained slides. FFPE tissue blocks and stained slides will be stored at room temperature, and unstained slides will be vacuum sealed and banked in refrigerated storage until processing.

H&E stained slides will undergo a pathology QA review to assess tumor content and annotate for macrodissection, when needed. Following macrodissection, tumor tissue from unstained slides will be scraped for co-extraction of DNA and RNA. The nucleic acids will be analyzed to determine concentration and quality prior to storage in a -80°C freezer. The remaining FFPE block and H&E stained slides will be stored at room temperature. Aliquots of RNA will be shipped for analysis.

* + - 1. Site(s) Performing Correlative Study

The assay will be performed at MoCha, Frederick National Laboratory for Cancer Research (FNLCR) under the supervision of Chris Karlovich, PhD.

* + - 1. Shipment of specimens from the EET Biobank to Site Performing Correlative Study

Specimens will be shipped from the EET Biobank to:

MoCha Lab, Frederick National Laboratory for Cancer Research (FNLCR)

1050 Boyles St.

Bldg. 459, Rm. 125

Frederick, MD 21702

Attn: Alyssa Chapman or Ruth Thornton

* + - 1. Contact Information for Notification of Specimen Shipment

Thomas Forbes, mochasamplereceiving@nih.gov

* + 1. Triapine PK (Blood)
			1. Specimen(s) Receipt and Processing at the Analytical Pharmacology Core, City of Hope

Frozen plasma will be received at the Analytical Pharmacology Core, City of Hope. Samples will be processed to assess triapine PK.

* + - 1. Site(s) Performing Correlative Study

This assay will be performed at the Analytical Pharmacology Core, City of Hope, under supervision of Tim Synold, PharmD.

* + - 1. Contact Information for Notification of Specimen Shipment

See Section 5.7.1.3.

* + 1. Triapine PK (CSF)
			1. Specimen(s) Receipt and Processing at the Analytical Pharmacology Core, City of Hope

Frozen CSF will be received at the Analytical Pharmacology Core, City of Hope. Samples will be processed to assess triapine PK.

* + - 1. Site(s) Performing Correlative Study

This assay will be performed at the Analytical Pharmacology Core, City of Hope, under supervision of Tim Synold, PharmD.

* + - 1. Contact Information for Notification of Specimen Shipment

See Section 5.7.1.3.

# TREATMENT PLAN

## Agent Administration

Treatment will be administered on an outpatient basis; the City of Hope sub-study will be administered on an inpatient basis for only select patients treated at City of Hope. Reported adverse events and potential risks are described in Section 10. Appropriate dose modifications are described in Section 7. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

|  |
| --- |
| **Dose Escalation Schedule** |
| **Dose Level** | **Dose\*** |
| **Triapine****(mg)\*\*** | **Radiation** |
| Level 1 (starting) | 50 | 35Gy in 10 fractions |
| Level 2 | 100 | 35Gy in 10 fractions |
| Level 3 | 150 | 35Gy in 10 fractions |
| Level 4 | 200 | 35Gy in 10 fractions |
| *\*Doses are stated as exact dose in units (*e.g.*, mg/m2, mcg/kg,* etc.*) rather than as a percentage.**\*\*Doses are administered once daily within 2 hours prior to radiation treatments.* |

|  |
| --- |
| **Regimen Description** |
| **Agent** | **Premedications; Precautions** | **Dose** | **Route** | **Schedule** | **Cycle Length** |
| Triapine | Fast (except for water) for 2 hours prior to dosing and for 1 hour after ingesting the oral dose\* | \*\* | Oral administration | Within 2 hours prior to radiation therapy  | 28 days (4 weeks) |
| Radiation | N/A | 35Gy in 10 fractions  | N/A | Once daily on weekdays |
| *\*Patients will be requested to maintain a medication diary of each dose of medication. The medication diary will be returned to clinic staff at the end of the course.**\*\*Doses as appropriate for assigned dose level.* |

* + 1. Triapine

Triapine is administered orally on an empty stomach (except for water 2 hours prior to dosing and 1 hour after ingesting). Patients will take triapine within 2 hours prior to radiation therapy, and thus will only take triapine on weekdays when radiation treatment is scheduled. Capsules should not be opened.

If a patient misses a scheduled dose of triapine, it is acceptable to take the missed dose within a window of 12 hours. If more than 12 hours have passed after the scheduled dose time, the missed dose should not be taken, and the patient should be instructed to take the next dose at the next scheduled time. If a patient vomits after taking their dose, they should not make up for this dose but should take the next scheduled dose. The patient will be requested to maintain a medication diary of each dose of medication ([Appendix D](#appendixD)). The medication diary will be returned to clinic staff at the end of each course.

* + 1. Definition of Intensity-Modulated Radiation Therapy (IMRT) Targeting and Treatment

For the purposes of this protocol, the term ‘intensity-modulated radiation therapy’ (IMRT) implies the targeting, planning, and aiming of radiation beams along any trajectory in 3-D space toward a target of known 3-D coordinates. The coordinate system is defined by reliable orthogonal alignment to in-treatment room lasers and patient body tattoos. Fiducial marker(s) may be used and located external or internal to the patient’s body. External fiducials may relate to a frame or treatment device. Internal fiducials may be implanted markers or reliably identifiable anatomy that is clearly visible on orthogonal kilovolt (KV) imaging inclusive of the tumor itself. In all cases, the relationship between the fiducial and the actual tumor position in real time should be reliably understood for both 3-D treatment planning and treatment delivery.

* + - 1. Radiation Therapy Prescription

Radiation will be administered in 10 daily fractions using IMRT (including volumetric modulated radiation therapy [VMAT] technique). The prescription dose per fraction is 3.5 Gy. The total prescription dose is 35 Gy. Radiation is scheduled on trial on weekdays for the first two weeks of the study.

* + - 1. Technical Factors for Radiation Therapy
				1. Physical Factors

Only photon (x-ray) beams with photon energies greater than or equal to 6MV are allowed for external beam radiation therapy. Cobalt-60 or charged particle beams (including electrons, proton, and heavier ions) are not allowed on this protocol. Electron beamsare notpermissible in this protocol.

Most commercially available photon-producing treatment platforms are allowed. Conventional linear accelerators or specialized linear accelerators with image guidance (e.g., Novalis, Trilogy, Synergy, Artiste, TrueBeam) are allowed. These units can be used with conformal dose delivery or IMRT. Specialized dose-painting accelerators (such as Cyberknife, Tomotherapy, etc.) are allowed provided that the units meet the technical specifications of the protocol and are used in a fashion that passes any credentialing required by the protocol. Conventional linear accelerators without add-on image-guided radiation therapy (IGRT) must have some other IGRT capability such as orthogonal 2-dimensional KV or CT-on-rails in the treatment room.

IMRT (including VMAT) are all acceptable planning techniques. IMRT is regarded here as a 3-D conformal treatment. IMRT can result in dosimetric inaccuracies especially in circumstances where tumor motion is either unknown or not properly accounted. When required for successful compliance, IMRT should only be utilized if tumor motion is less than five millimeters (5 mm), or, if motion management inherently diminishes motion effects (e.g., gating, breath hold, or tracking). Planning techniques may differ for each lesion to be treated provided that the tumor motion is properly accounted for with each technique.

* + - * 1. Minimum Field Aperture (Field Size) Dimension for IMRT Treatment

Because of uncertainties in beam commissioning resulting from electronic disequilibrium within small beam apertures, the minimum field size should not be smaller than 2cm x 2cm. The prescription dose is prescribed to the edge of the defined planning treatment volume (PTV).

* + - * 1. Patient Positioning

Patients will be positioned in a stable position for accurate reproducibility of the target position during treatment and between treatments. Positions uncomfortable for the patient should be avoided so as to minimize uncontrolled movement during treatment. Immobilization must be reliable to ensure that the gross tumor volume (GTV) does not deviate beyond the confines of the PTV. All patients will be positioned with rigid immobilization and daily image guidance to ensure positioning accuracy and a magnitude that justifies the PTV margin applied.

* + - 1. Radiation Simulation and Planning

The patient will undergo a treatment planning simulation. All patients will be immobilized using customized devices like a fitted mask (conforming to patients’ external contours) with reference to the referent coordinate system per institutional routine in order to prevent any inadvertent patient motion during external beam radiation treatment. An MRI and/or CT scan will be obtained with the patient in the immobilized treatment position and required for treatment planning.

CT scan range must allow simultaneous view of the patient anatomy and any fiducial system for tumor targeting (if used) and be adequate to ensure contouring of all targeted cancers, as well as necessary organs at risk (OAR), as defined below. High-resolution CT scans should be obtained with uniform slice thickness of less than or equal to three millimeters (≤3 mm).

The target lesion(s) should be outlined by an appropriately trained physician and designated the gross tumor volume (GTV). The GTV will be defined using contrast-enhanced CT and/or MRI images. For patients who underwent resection for recurrent disease and there is no residual enhancing tumor noted, the post-operative resection cavity will be outlined. No additional clinical target volume (CTV) expansion is required. An expansion of no more than 5 mm is optional for lesions measuring less than 3.5cm in maximal diameter and must be reported when used. A planning target volume (PTV) expansion is based on image guidance and immobilization. Regardless of methods, the PTV expansion should be between 3-5 mm or per institutional standards.

* + - 1. Radiation Treatment Planning Technique
				1. Planning Parameters

Multiple, non-coplanar beam arrangements or arc-based therapy is advised. When static beams are used, a minimum of four radiation beams should be used (but other techniques are permissible if dose constraints are met). For arc rotation techniques, a minimum of 340 degrees (cumulative for all beams) should be utilized. The minimum field size used should be consistent with the minimum field size commissioned for use at the institution and should not be smaller than 2cm x 2cm. Prescription lines covering the PTV will typically range between 95-100% line; however, higher isodose line hotspots must be manipulated to occur within the target and not in adjacent normal tissue.

**Planning Priorities:** Every attempt should be made to successfully satisfy all of the planning goals and OAR criteria without deviation. In some circumstances, it may not be possible to meet all the ideal criteria leading to plans with an acceptable deviation. Special consideration should be given to avoid exit dose through the oral cavity and mucosa as well as avoid doses greater than the prescription dose within the scalp.

**Organs at Risk (OAR):** Protocol-specific OARs that should be contoured are listed below. A minimum planning risk volume (PRV) expansion of 3mm is recommended for optic chiasm and optic nerves:

* Brain
* Brainstem
* Optic chiasm
* Optic nerves
	+ - 1. Radiation Planning Goals and Dose Constraints

Two scenarios for normal tissue limits are considered: (1) No previous radiation to the local area or OAR (2) Previous radiation to the local area or OAR. The OAR limits for both scenarios are provided in the following table.

| Structures | Goal | Acceptable | Avoidance Endpoint |
| --- | --- | --- | --- |
| Optic nerves and optic chiasm PRV | No prior radiation: D0.03cc ≤ 35Gy Prior radiation: D0.03cc ≤ 20Gy | No prior radiation: D0.03cc > 35Gy but ≤ 36.75Gy Prior radiation: D0.03cc > 20Gy but ≤ 25Gy | Neuritis |
| Brainstem | No prior radiation: D0.03cc ≤ 35Gy Prior radiation: D0.03cc ≤ 24Gy | No prior radiation: D0.03cc > 35Gy but ≤ 36.75Gy Prior radiation: D0.03cc > 24Gy but ≤ 30Gy | Cranial neuropathies |

* + - 1. Online Treatment Image-Guided Localization and Treatment

IMRT image-guided radiation therapy (IGRT) is a preferred delivery modality on this protocol. This is accomplished with standard treatment planning with position verification using a cone beam CT (CBCT) scan. A CBCT is a CT scan taken of the patient and target structure of interest while the patient is immobilized on the treatment table. During the treatment, the patient is immobilized exactly as was done for the simulation. The patient is then set-up in the treatment position according to laser-guiding in the treatment room.

All radiation treatments will be delivered whenever possible. If treatment must be terminated prematurely on any fractions of one to ten, compensate as follows: If 2/3 or more of all non-zero segments were delivered, then the untreated segments plus the full next fraction should be treated on the next treatment day (this should introduce an error of < 5% in biologic effective dose delivered). If fewer than 2/3 of the non-zero segments were treated, then the untreated portion of this fraction (only) will be made up for on the following interval day. If treatment must be terminated prematurely, and 90% of the non-zero segments were treated, then no further treatment shall be given (this should introduce an error of < 5% for total biologic effective dose delivered). If fewer than 90% of the non-zero segments were treated, then the deficit shall be delivered on the following day. All such variations shall be recorded.

## Definition of Dose-Limiting Toxicity

Dose-limiting toxicities (DLTs) will be graded in severity according to the guidelines outlined in the NCI CTCAE version 5.0, graded 1 to 5 with grade 5 toxicity meaning death. The DLT window will be 28 days after treatment initiation. A DLT is defined as clinically significant grade 3 or 4 toxicity lasting more than 24 hours that is possibly, probably, or definitely related to study treatment, including:

| **Adverse Event** | **Grade** |
| --- | --- |
| Anemia | ≥ 4 |
| Febrile neutropenia | ≥ 4 |
| White blood cell decreased | ≥ 4 |
| Hemolysis | ≥ 4 |
| Platelet count decreased | ≥ 4 |
| Methemoglobinemia | ≥ 4 |
| Cyanosis | ≥ 3 |
| Left ventricular systolic dysfunction | ≥ 3  |
| Nausea | ≥ 3 (uncontrollable) |
| Vomiting | ≥ 3 (uncontrollable) |
| Colitis | ≥ 3 |
| Constipation | ≥ 3 |
| Diarrhea | ≥ 3 |
| Dyspepsia | ≥ 3 |
| Fatigue | ≥ 3 (lasting greater than 1 week) |
| Fever | ≥ 4 |
| Chills | ≥ 3 |
| Infection | ≥ 3 |
| Alanine aminotransferase increased  | ≥ 3 |
| Alkaline phosphatase increased | ≥ 3 |
| Aspartate aminotransferase increased  | ≥ 3 |
| Blood bilirubin increased | ≥ 3 |
| Creatinine increased | ≥ 3 |
| Lipase increased | ≥ 3 |
| Blood bicarbonate decreased | ≥ 3 (that cannot be corrected to ≤2 within 72 hours) |
| Hyponatremia  | ≥ 3 (that cannot be corrected to ≤2 within 72 hours) |
| Hypercalcemia | ≥ 3 (that cannot be corrected to ≤2 within 72 hours) |
| Hyperkalemia | ≥ 3 (that cannot be corrected to ≤2 within 72 hours) |
| Hypoalbuminemia | ≥ 3 (that cannot be corrected to ≤2 within 72 hours) |
| Dehydration | ≥ 3  |
| Anorexia | ≥ 3  |
| Weight loss | ≥ 3  |
| Myalgia | ≥ 3 |
| Dizziness | ≥ 3 |
| Dysgeusia | ≥ 3 |
| Headache | ≥ 3 |
| Cough | ≥ 3 |
| Dyspnea | ≥ 3 |
| Hypoxia | ≥ 3 |
| Pneumonitis | ≥ 3 |
| Rash | ≥ 3 |
| Dermatitis radiation | ≥ 4 |
| Flushing | ≥ 3 |
| Hypertension | ≥ 3 |
| Hypotension | ≥ 3 |

Triapine has been observed to alter iron II (Fe2+) in hemoglobin and creates Fe3+ methemoglobin, an inactive state that cannot deliver oxygen to tissues. Fe2+ in hemoglobin auto-oxidizes to Fe3+ methemoglobin at a rate of ~3% per day and is counterbalanced by a reductase system that limits methemoglobin concentrations to <1% of hemoglobin. Triapine may disrupt this balance and lead to higher-than-normal methemoglobin levels resulting in dyspnea, headaches, and altered cognition. Should a patient develop grade 3 dyspnea lasting longer than 24 hours, arterial or venous blood gas (serial sampling as clinically indicated) will be obtained. Management will include supplemental oxygen and IV administration of methylene blue to dose of 1-2mg/kg over 5-30 minutes (maximum single dose 100mg). Methylene blue administration can be repeated 1 hour later if methemoglobin levels remain greater than 30% or symptoms persist.

Management and dose modifications associated with the above adverse events are outlined in Section 7.

Dose escalation will proceed within each cohort according to the scheme in Section 9.1.1. Dose-limiting toxicity (DLT) is defined above.

We will employ the Bayesian optimal interval design with backfill (BF-BOIN) (Zhao *et al.,* 2024) to guide dose escalation and establish the MTD. BF-BOIN enables backfilling patients to dose levels that are cleared for safety and have demonstrated activity during the dose escalation, thereby generating additional data on safety, tolerability, and preliminary activity on doses below the MTD. This facilitates the identification of the recommended phase 2 dose (RP2D). The procedure is fully described in Section 9.

## General Concomitant Medication and Supportive Care Guidelines

There are no known drug interactions with triapine. Because there is a potential for interaction of triapine with other concomitantly administered drugs, the case report form must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies. The Principal Investigator should be alerted if the patient is taking any agent known to affect or with the potential for drug interactions, including bevacizumab for symptom control and supportive care. The study team should check a frequently updated medical reference for a list of drugs to avoid or minimize use of. [Appendix C](#_APPENDIX_C_PATIENT) (Patient Drug Interactions Handout and Wallet Card) should be provided to patients if available.

Patients may use bevacizumab for the purpose of symptom control.

FDA-approved biosimilar growth factors are acceptable according to institutional policies.

## Duration of Therapy

In the absence of treatment delays due to adverse event(s), treatment may continue until one of the following criteria applies:

* Disease progression
* Intercurrent illness that prevents further administration of treatment
* Unacceptable adverse event(s)
* Patient decides to withdraw from the study
* General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
* Clinical progression
* Patient non-compliance
* Pregnancy
* All women of child-bearing potential should be instructed to contact the investigator immediately if they suspect they might be pregnant (*e.g.*, missed or late menstrual period) at any time during study participation.
* The investigator must immediately notify CTEP in the event of a confirmed pregnancy in a patient participating in the study.
* Termination of the study by sponsor
* The drug manufacturer can no longer provide the study agent

The reason(s) for protocol therapy discontinuation, the reason(s) for study removal, and the corresponding dates must be documented in the Case Report Form (CRF).

## Duration of Follow-Up

Patients will be checked at the clinic approximately two weeks after completing radiation treatment and then followed (at the clinic or by telemedicine) for every three months for up to two years or until removal from study, disease progression, or death, whichever occurs first. Patients removed from study for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event.

# DOSING DELAYS/DOSE MODIFICATIONS

|  |  |
| --- | --- |
| **Dose Level** | **Triapine (mg)** |
| 1 (starting) | 50, once daily, weekdays |
| 2 | 100, once daily, weekdays |
| 3 | 150, once daily, weekdays |
| 4 | 200, once daily, weekdays |

Radiation therapy may only be suspended secondary to a significant adverse event at the joint discretion of the patient’s treating physicians and the principal investigator.

## Recommended Dose Modifications for Triapine

| **Nausea** | **Management/Next Dose for Triapine** | **Management/Next Dose for Radiation** |
| --- | --- | --- |
| ≤ Grade 1 | No change in dose | No change in dose |
| Grade 2 | Hold until ≤ Grade 1. Resume at same dose level. | Consider hold until ≤ Grade 1. Resume next fraction at same dose.  |
| Grade 3 | Hold\* until < Grade 2. Resume at one dose level lower, if indicated.\*\* | Hold\* until < Grade 2. Resume next fraction at same dose. |
| Grade 4 | Off protocol therapy | Off protocol therapy |
| \*Patients requiring a delay of >2 weeks should go off protocol therapy.\*\*Patients requiring > two dose reductions should go off protocol therapy. |
| Recommended management: antiemetics. |

| **Vomiting** | **Management/Next Dose for Triapine** | **Management/Next Dose for Radiation** |
| --- | --- | --- |
| ≤ Grade 1 | No change in dose | No change in dose |
| Grade 2 | Hold until ≤ Grade 1. Resume at same dose level. | Consider hold until ≤ Grade 1. Resume next fraction at same dose. |
| Grade 3 | Hold\* until < Grade 2. Resume at one dose level lower, if indicated.\*\* | Hold\* until < Grade 2. Resume next fraction at same dose. |
| Grade 4 | Off protocol therapy | Off protocol therapy |
| \*Patients requiring a delay of >2 weeks should go off protocol therapy.\*\*Patients requiring > two dose reductions should go off protocol therapy. |
| Recommended management: antiemetics. |

| **Diarrhea** | **Management/Next Dose for Triapine** | **Management/Next Dose for Radiation** |
| --- | --- | --- |
| ≤ Grade 1 | No change in dose | No change in dose |
| Grade 2 | Hold until ≤ Grade 1. Resume at same dose level. |  Consider hold until ≤ Grade 1. Resume next fraction at same dose.  |
| Grade 3 | Hold\* until < Grade 2. Resume at one dose level lower, if indicated.\*\* | Hold\* until < Grade 2. Resume next fraction at same dose. |
| Grade 4 | Off protocol therapy | Off protocol therapy |
| \*Patients requiring a delay of >2 weeks should go off protocol therapy.\*\*Patients requiring > two dose reductions should go off protocol therapy. |
| Recommended management: Loperamide antidiarrheal therapy.Dosage schedule: 4 mg at first onset, followed by 2 mg with each loose motion until diarrhea-free for 12 hours (maximum dosage: 16 mg/24 hours).Adjunct anti-diarrheal therapy is permitted and should be recorded when used. |

| **Neutropenia** | **Management/Next Dose for Triapine** | **Management/Next Dose for Radiation** |
| --- | --- | --- |
| ≤ Grade 1 | No change in dose | No change in dose |
| Grade 2 | Hold until ≤ Grade 1. Resume at same dose level. | Consider hold until ≤ Grade 1. Resume next fraction at same dose. |
| Grade 3 | Hold\* until < Grade 2. Resume at one dose level lower, if indicated.\*\* | Consider hold until ≤ Grade 1. Resume next fraction at same dose. |
| Grade 4 | Off protocol therapy |  Hold until ≤ Grade 3\*\*\* or off protocol therapy |
| \*Patients requiring a delay of >2 weeks should go off protocol therapy.\*\*Patients requiring > two dose reductions should go off protocol therapy.\*\*\* Consider temporary hold of radiation until absolute neutrophil count recovers to > 500 / mm3 at discretion of treating physician. |
| Recommended management: G-CSF is allowed.  |

| **Thrombocytopenia** | **Management/Next Dose for Triapine** | **Management/Next Dose for Radiation** |
| --- | --- | --- |
| ≤ Grade 1 | No change in dose | No change in dose |
| Grade 2 | Hold until ≤ Grade 1. Resume at same dose level. | Consider hold until ≤ Grade 1. Resume next fraction at same dose. |
| Grade 3 | Hold\* until < Grade 2. Resume at one dose level lower, if indicated.\*\* | Consider hold until ≤ Grade 1. Resume next fraction at same dose. |
| Grade 4 | Off protocol therapy | Hold until ≤ Grade 3\*\*\* or off protocol therapy |
| \*Patients requiring a delay of >2 weeks should go off protocol therapy.\*\*Patients requiring > two dose reductions should go off protocol therapy.\*\*\* Consider temporary hold of radiation until platelet count recovers to > 50,000 / mm3 at discretion of treating physician. |
| Recommended management: Platelet transfusions are allowed. |

* + 1. Methemoglobinemia

It is expected for all patients to show a transient rise in methemoglobin (up to 3-5%, maximum 15%) while on study. Please note, it is the trend in the O2 saturation that is of importance. Since pulse oximetry is known to be unreliable in the presence of significant methemoglobinemia, weight should not be given to a single value alone. In any case where there is significant doubt, serial spot methemoglobins should be obtained (as clinically indicated, based on symptoms such as but not limited to skin discoloration, cyanosis, coma, or dysrhythmia and levels can be repeated every 6-8 hours until levels <20%) and consultation as needed.

If the patient remains asymptomatic *or* methemoglobin levels <5% within 24 hours, no changes to treatment or dose are required. If patient is symptomatic (*e.g.,* dyspnea *or* hypoxia [<92%] requiring oxygen), obtain “spot” methemoglobin level and serial sampling as clinically indicated (based on symptoms such as but not limited to skin discoloration, cyanosis, coma, dysrhythmia, levels can be repeated every 6-8 hours until levels <20%). Supportive care with supplemental oxygen starting at 2 liters / minute should be provided as clinically indicated.

For patients not fitting this pattern, the following guidelines should be followed:

* **IF** methemoglobin is asymptomatic, <20%, and NOT accompanied by (oxygen saturation <92%) hypoxia *or* methemoglobin levels < 5% within 24 hours, **THEN**: treat without change in dose.
* **IF** methemoglobin >15% lasts more than 3 hours OR if methemoglobin >20% OR if oxygen saturation <92% OR patient has moderate to severe symptoms, **THEN**: provide appropriate supportive care and obtain arterial blood gases which can be repeated every 6-8 hours. A pO2 <80 should result in hospitalization and will be counted as DLT. If pO2 normalizes within 24 hours, retreatment at a lower dose level may be considered by the Principal Investigator.
* **IF** patient has moderate to severe, but rapidly reversible (i.e., over several hours) symptoms NOT requiring hospitalization, **THEN** retreatment at a lower dose level may be considered by the Principal Investigator.
* **IF** patient develops hypotension (systolic blood pressure < 85 mmHg), **THEN** triapine administration should be stopped and not receive additional triapine treatment on study.

Treatment options for methemoglobinemia could include methylene blue, 1-2 mg/kg IV over five minutes. However, methylene blue is contraindicated in patients with glucose-6-phosphate (G6PD) deficiency, since its pharmacologic action as an electron carrier in the reduction of methemoglobin is itself dependent on the generation of NADPH by G6PD through the hexose monophosphate shunt. Thus, methylene blue may be at best ineffective in such patients and may have the potential to complicate the clinical situation by provoking hemolysis, although this association is less clear. In situations where the use of methylene blue may be contraindicated (*e.g*., in those individuals who are in the high-risk group [patients of African, Asian or Mediterranean origin/ancestry], who may have had a prior false negative G6PD deficiency test, the successful use of ascorbic acid (1000 mg IV q6h) has been described.

# PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational agent administered in this study can be found in Section 10.1.

## CTEP IND Agent

* + 1. Triapine (NSC 663249)

**Chemical Name:** 3-aminopyridine-2-carboxaldehyde thiosemicarbazone

**Other Names:** 3-AP

**Classification:** Triapine**,** an α-heterocyclic carboxaldehyde thiosemicarbazone (HCT), is a ribonucleotide reductase (RNR)inhibitor that acts on the M2 (R2) subunit. The HCTs are the most potent RNR inhibitors, being 65 -5,000 times more potent than hydroxyurea.

**Mechanism of Action:** Ribonucleotide reductase (RNR) inhibitor

**CAS Registry Number:** 143621-35-6

**Molecular Formula:** C7H9N5S **M.W.:** 195

**Approximate Solubility:** Water = 0.1 mg/mL

 Ethanol = 1.25 mg/mL

 PEG-300 = 15 mg/mL

**How Supplied:**  Triapinecapsulesare supplied by Nanopharmaceutics, LLC and distributed by the CTEP, DCTD, NCI. Each Triapine capsule contains 50 mg of Triapine in combination with Starch 1500 and Magnesium Stearate in a size 1, hard gelatin, white, opaque capsule. Each bottle contains 30 capsules.

**Storage:** Store Triapinecapsulesat room temperature 25ºC, excursions permitted to 15ºC to 30ºC.

If a storage temperature excursion is identified, promptly return Triapinecapsules to between 15 and 30°C and quarantine the supplies. Provide a detailed report of the excursion (including documentation of temperature monitoring and duration of the excursion) to PMBAfterHours@mail.nih.gov for determination of suitability.

**Stability:** Shelf-life stability studies of Triapinecapsulesare on-going.

**Route** **of Administration:** Oral administration. Patients will be asked to fast (except for water) for 2 hours prior to dosing and for 1 hour after ingesting the oral dose. Capsules should not be opened.

**Availability**

Triapine is an investigational agent supplied to investigators by the Division of Cancer Treatment and Diagnosis (DCTD), NCI.

Triapine is provided to the NCI under a Collaborative Agreement between the Pharmaceutical Collaborator and the DCTD, NCI (see Section 13.5).

* + 1. Agent Ordering and Agent Accountability
			1. NCI-supplied agents may be requested by eligible participating Investigators (or their authorized designee) at each participating institution. The CTEP-assigned protocol number must be used for ordering all CTEP-supplied investigational agents. The eligible participating investigators at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA Form 1572 (Statement of Investigator), NCI Biosketch, Agent Shipment Form, and Financial Disclosure Form (FDF). If there are several participating investigators at one institution, CTEP-supplied investigational agents for the study should be ordered under the name of one lead participating investigator at that institution.

*[The CTEP Pharmaceutical Management Branch (PMB) will provide direction as to when sites can order PMB-supplied agents.]*

Submit agent requests through the PMB AURORA application. Access to AURORA requires the establishment of credentials necessary to access secure NCI Clinical Oncology Research Enterprise (CORE) systems, maintenance of an “active” account status, a “current” password, and active person registration status. For questions about drug orders, transfers, returns, or accountability, call or email PMB any time or use the dialog function in AURORA to communicate with PMB staff. Refer to the PMB’s website for specific policies and guidelines related to agent management.

* + - 1. Agent Inventory Records – The investigator, or a responsible party designated by the investigator, must maintain a complete accountability of the receipt, dispensing and final disposition of all agents received from the PMB using the appropriate NCI Investigational Agent (Drug) Accountability Record (DARF) available on the CTEP forms page. Store and maintain separate NCI Investigational Agent Accountability Records for each agent, strength, formulation and ordering investigator on this protocol.

Product Quality Complaint (PQC): A product quality complaint is defined as any suspicion of a product defect related to a potential quality issue during manufacturing, packaging, release testing, stability monitoring, dose preparation, storage or distribution of the product, or delivery system. Not all PQCs involve a study subject. Lot or batch numbers are of high significance and need to be provided where and when possible. PQC must be reported to the PMB as soon as the PQC is identified. Report PQC to PMB at PMBAfterHours@mail.nih.gov or by using the dialog function in AURORA to communicate with PMB staff.

* + 1. Material Safety Data Sheets
* The current versions of the material safety data sheets (MSDS or SDS) for PMB-distributed agents will be accessible to site investigators and research staff through the PMB AURORA application. Questions about MSDS access may be directed to the PMB at PMBAfterHours@mail.nih.gov or by using the dialog function in AURORA to communicate with PMB staff.
	+ 1. Investigator Brochure Availability

The current versions of the IBs for the agents will be accessible to site investigators and research staff through the PMB AURORA application. Access to AURORA requires the establishment of credentials necessary to access secure NCI Clinical Oncology Research Enterprise (CORE) systems, maintenance of an “active” account status, a “current” password and active person registration status. Questions about IB access may be directed to the PMB IB Coordinator via email.

* + 1. Useful Links and Contacts
	+ CTEP Forms, Templates, Documents: <http://ctep.cancer.gov/forms/>
	+ NCI CTEP Investigator Registration: RCRHelpDesk@nih.gov
	+ PMB policies and guidelines: <http://ctep.cancer.gov/branches/pmb/agent_management.htm>
	+ PMB AURORA application: <https://ctepcore.nci.nih.gov/aurora/login>
	+ CTEP Identity and Access Management (IAM) account: <https://ctepcore.nci.nih.gov/iam/>
	+ CTEP IAM account help: ctepreghelp@ctep.nci.nih.gov
	+ IB Coordinator: IBCoordinator@mail.nih.gov
	+ PMB email: PMBAfterHours@mail.nih.gov
	+ PMB phone and hours of service: (240) 276-6575 Monday through Friday between 8:30 am and 4:30 pm (ET)

# STATISTICAL CONSIDERATIONS

## Study Design/Endpoints

This is a phase 1 trial to evaluate the safety and efficacy of adding the RRM2 inhibitor, triapine, to radiation therapy for recurrent GBM. We will employ the BF-BOIN design to guide dose escalation and establish the MTD (Zhao *et al.,* 2024). BF-BOIN enables backfilling patients to dose levels that are cleared for safety and have demonstrated activity during the dose escalation, thereby generating additional data on safety, tolerability, and preliminary activity on doses below the MTD. This facilitates the identification of the RP2D. Patients with recurrent GBM will be eligible. Patients will receive radiation therapy in combination with oral triapine administered once daily within two hours prior to radiation for two weeks according to the dose level schedule (Section 6.1).

**Primary Objective:**

1. To identify the safety and MTD of oral triapine used in combination with radiation therapy for patients with recurrent GBM. The incidence of DLTs and any AEs will be assessed.

**Primary Hypothesis:** We hypothesize that triapine can be safely administered in conjunction with radiation therapy for patients with recurrent GBM.

**Secondary Objectives:**

1. To observe and record anti-tumor activity. The objective response status will be measured and recorded. The objective response rate (ORR) is based on Response Assessment in Neuro-Oncology Criteria for High- and Low-Grade Gliomas in Adults (RANO 2.0).
2. To determine the pharmacokinetics of oral triapine in plasma and the CNS.
3. To evaluate the efficacy of triapine when administered in combination with radiation therapy by assessing:
* PFS
	+ Measured by time to disease progression or death from any cause.
* OS
	+ Measured by time to death from any cause.
* The proportion of patients requiring bevacizumab for symptom control
	+ Measured by the number of patients who initiated bevacizumab during study period.
* The correlation of genetic mutations in select genes (*e.g., p53*, *p16*, *KRAS*, and *Pi3k/mTOR/AKT*) with tumor response and clinical outcomes
	+ Genetic mutations detected on whole exome sequencing from tumor tissue

**Secondary Hypotheses:** We hypothesize that the pharmacokinetics of oral triapine in GBM patients is comparable to historical controls, and triapine crosses the human blood-brain barrier. We also hypothesize that the addition of triapine will prolong median PFS and median OS relative to historical controls without increasing the proportion of patients requiring bevacizumab for symptom control. We hypothesize that there is a correlation with genetic mutations in select genes (*e.g., p53, p16, KRAS,* and *Pi3k/mTOR/AKT*) with tumor response and clinical outcomes.

* + 1. Study Design

The BF-BOIN consists of two interrelated components: dose escalation and backfill.

**Dose escalation**

The maximum sample size of the dose escalation is 30. Patients are enrolled and treated in cohorts of three patients at a time, starting at dose level 1. The target toxicity rate for the MTD is *ϕ*= 0.25. DLTs are defined in Section 6.2, and only those DLTs that occur within 28 days after treatment initiation will be used to guide dose escalation. BF-BOIN employs the BOIN rule (Yuan *et al.,* 2016) to guide dose escalation and de-escalation, with additional rules to address potential conflicts between dose escalation data and backfill data (*e.g.,* backfill data later suggest that a dose previously cleared for safety by dose escalation is toxic) as described later.

**Figure 5** shows the rule to guide dose escalation and de-escalation in the BF-BOIN design. The rule is optimized to minimize the probability of incorrect dose assignment. For the purpose of overdose control, doses $j$ and higher levels will be eliminated from further examination if Pr$(p\_{j}>$ 0.25 | data) $>$ 0.95 and at least three evaluable patients have been treated at dose level $j$, where $p\_{j}$ is the true DLT rate of dose level $j,j=$ 1,$\cdots $, 4. This posterior probability is evaluated based on the beta-binomial model $y\_{j}∣p\_{j}∼binomial\left(p\_{j}\right)$ with $p\_{j}∼uniform\left(0,1\right)$, where $y\_{j}$ is the number of patients experienced DLT at dose level $j$. When the lowest dose is eliminated, stop the trial for safety. The probability cutoff 0.95 is chosen to be consistent with the common practice that when the target DLT rate <=1/6, a dose with 2/3 patients experienced DLT is eliminated.



**Figure 5.** Flowchart for trial conduct using the BOIN design.

The above dose escalation/de-escalation and elimination rule can be equivalently presented in **Table 1** as follows, which will be used to conduct the trial:

Specifically, let *c* denote the dose level used for treating the current dose-escalation cohort. The steps to conduct the dose escalation in BF-BOIN are described as follows:

1. Patients in the first cohort are treated at dose level 1.
2. Use the BOIN rule displayed in Table 1 to assess whether the decision based on the data observed at the current dose *c* conflicts with that based on the data observed at the backfill dose (or any backfill doses if more than one dose is backfilled). The conflict is defined in **Table 2**.
3. (i) If no conflict is identified, perform dose escalation/de-escalation according to Table 1. When using Table 1, please note the following:
4. “Eliminate” means eliminating the current dose and higher doses from the trial to prevent treating any future patients at these doses because they are overly toxic.
5. When the lowest dose is eliminated, stop the trial for safety. In this case, no dose should be selected as the MTD.
6. If the current dose *c* is the lowest dose and the rule indicates dose de-escalation, treat the new patients at the lowest dose unless the number of DLTs reaches the elimination boundary, at which point terminate the trial for safety.
7. If the current dose is the highest dose and the rule indicates dose escalation, treat the new patients at *c*.

(ii) If conflict is observed, let *b*\* (*b*\*< *c*) denote the backfill dose where the conflict occurs, and let *m* and *t* respectively denote the total number of patients who completed DLT assessment and the total number of patients who experienced DLT, pooled from *b*\* to the current dose *c*. Based on the pooled data m and *t*, perform dose escalation/de-escalation according to Table 1, using the same rules described in Step 3(i) with the following modification:

1. If the decision according to Table 1 is to de-escalate the dose, rather than simply de-escalating to *c*-1, de-escalate to the highest dose level *k* that is deemed safe with $\hat{q}\_{k}\leq $ 0.298, where $b^{\*}\leq k\leq c-1$. If such a dose does not exist, de-escalate to $b^{\*}-1$. Here, $\hat{q}\_{k}$ is the pooled DLT rate from *b*\* to a higher dose *k* (*k* ≥ *b*\*), defined as:
$ \hat{q}\_{k}=\frac{the total number of patients experienced DLT from dose b^{\*} to k}{the total number of patients completed DLT assessment from dose b^{\*} to k}.$

In the case that the elimination boundary is crossed, de-escalate to *b*\*-1.
2. Repeat step 2 and 3 until the maximum dose escalation sample size of 30 is reached, or stop the trial early when one of the following two conditions is satisfied:
3. The lowest dose reaches the elimination boundary, and if there is a conflict, the decision based on the pooled data using rule 3e is to de-escalate below the lowest dose. In this scenario, no dose should be selected as the MTD.
4. The number of patients treated at the current dose >=12 and the decision according to step 3 is to stay at the current dose.

**Table 1.** Dose Escalation/De-Escalation Rule for the BF-BOIN Design.

| Decision | The number of evaluable patients treated at a current dose |
| --- | --- |
| **3** | 4 | 5 | **6** | 7 | 8 | **9** | 10 | 11 | **12** |
| Escalate if # of DLT <= | **0** | 0 | 0 | **1** | 1 | 1 | **1** | 1 | 2 | **2** |
| Stay if # of DLT = | **1** | 1 | 1 | **NA** | 2 | 2 | **2** | 2 | 3 | **3** |
| De-escalate if # of DLT >= | **NA** | 2 | 2 | **2** | 3 | 3 | **3** | 3 | 4 | **4** |
| Eliminate if # of DLT >= | **2** | 3 | 3 | **3** | 4 | 4 | **4** | 5 | 5 | **5** |

Note: “# of DLT” is the number of patients with at least one DLT. “NA” is not applicable.

**Table 2.** Conflicting Decisions Between the Current Dose of Dose Escalation and Backfilling Doses.

|  |  |
| --- | --- |
|  | Decision according to the data observed at the current dose *c* |
| Decision according to the data observed at a backfilling dose *b* | Escalation | Stay | De-escalation or elimination |
| Escalation | no conflict | no conflict | no conflict |
| Stay | conflict | no conflict | no conflict |
| De-escalation or elimination | conflict | conflict | conflict |

**Backfilling**

BF-BOIN adaptively opens and closes a dose for backfilling based on observed data as follows. A dose level $b$ is regarded as eligible for backfilling if it satisfies the following two conditions:

* (Cleared for safety) $b$ is lower than the current dose of the dose escalation (i.e., $b<c$).
* (Demonstrate activity) At least one response is observed at $b$ or at a dose lower than $b$.

Dose level $b$ will be closed for backfilling if (a) the number of evaluable patients treated at $b$ is $\geq $ 12, or (b) both of the following two conditions are met:

* The observed DLT rate based on all cumulative patients completed DLT assessment at $b$ is greater than the de-escalation boundary $λ\_{d}$= 0.298, and
* the pooled DLT rate based on the pooled DLT data over $b$ and $b+1$ is also greater than $λ\_{d}$= 0.298.

The requirement to examine the pooled ($b$ and $b+1$) DLT rate is needed to reduce the risk of accidentally closing backfilling caused by the variation associated with small sample sizes at $b$. When $b$ is closed for backfilling due to rule (b), the doses higher than $b$ should also be closed for backfilling. A dose closed to backfilling for toxicity may later be re-opened for backfilling when accruing toxicity data indicate that the dose is actually safe and the backfill closing conditions no longer hold. Dose $b$ is permanently closed for backfilling if the number of evaluable patients treated at $b$ is $\geq $ 12.

In the dose escalation component of BF-BOIN, patient enrollment is staggered between cohorts. This means that the next cohort cannot commence enrollment until all patients of the current dose-escalation cohort have completed DLT assessment. In contrast, for the backfilling component of BF-BOIN, no staggering is necessary, and available patients can be promptly enrolled and assigned to an open backfilling dose. Accordingly, BF-BOIN assigns the next available patient as follows:

* If the current cohort of the dose escalation has not been filled, the patient will be allocated to that dose-escalation cohort;
* Otherwise, the patient will be allocated to a dose that is open for backfilling. If multiple dose levels are open for backfilling, the patient will be assigned to the highest one.

The backfilling ends once the dose escalation is ended.

After the trial is completed, the MTD is determined with all the data based on isotonic regression by the shiny app “BF-BOIN” available at <http://www.trialdesign.org>. Specifically, the MTD is the dose for which the isotonic estimate of the toxicity rate is closest to the target toxicity rate. If there are ties, the higher dose level will be selected when the isotonic estimate is lower than the target toxicity rate, and the lower dose level will be selected when the isotonic estimate is greater than or equal to the target toxicity rate.

At the conclusion of the trial, all available safety data will be reviewed. If a significant number of clinically significant adverse events outside the definition of DLT occurred at the MTD, the RP2D may be a lower dose level than the MTD. Examples of clinically significant events that would be considered are those events like DLTs but occurred in later cycles outside the DLT window, treatment discontinuation due to an adverse event attributed to triapine, or patient withdrawal from the study due to intolerable adverse events.

**Table 3** shows the operating characteristics of the trial design based on 1000 simulations of the trial using shiny app “BF-BOIN” (BF-BOIN v1.0.3.0) available at <http://www.trialdesign.org>. The operating characteristics show that the design selects the true MTD, if any, with high probability and allocates a high percentage of patients to the dose levels with the DLT rate closest to the target of 0.25.

**Table 3.** Operating Characteristics of the BF-BOIN Design

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
|  | **Dose 1** | **Dose 2** | **Dose 3** | **Dose 4** | **# Patients** | **% Early Stopping** |
| Scenario 1 |  |  |  |  |  |  |
| True DLT rate | **0.25** | 0.42 | 0.5 | 0.59 |  |  |
| Response Rate | 0.25  | 0.42  | 0.5  | 0.59  |  |  |
| Selection % | 82.1  | 9.5  | 0.7  | 0  |  | 7.7 |
| % Pts Treated | 68  | 27.7  | 4  | 0.3  | 19.06 |  |
| Scenario 2 |  |  |  |  |  |  |
| True DLT rate | 0.1 | **0.25** | 0.4 | 0.62 |  |  |
| Response Rate | 0.1  | 0.25  | 0.4  | 0.62  |  |  |
| Selection % | 24.1  | 62.3  | 13.1  | 0.1  |   | 0.4 |
| % Pts Treated | 39.65 | 42.83 | 15.39 | 2.13  | 28.25 |  |
| Scenario 3 |  |  |  |  |  |  |
| True DLT rate | 0.02  | 0.1  | **0.25**  | 0.42  |   |  |
| Response Rate | 0.02  | 0.1  | 0.25  | 0.42  |   |  |
| Selection % | 0.3  | 22.4  | 66.7  | 10.6  |   | 0 |
| % Pts Treated | 16.33 | 35.71 | 35.61 | 12.35 | 31.34 |  |
| Scenario 4 |  |  |  |  |  |  |
| True DLT rate | 0.05  | 0.08  | 0.12  | **0.25**  |   |  |
| Response Rate | 0.05  | 0.08  | 0.12  | 0.25  |   |  |
| Selection % | 1.4  | 6.1  | 29  | 63.4  |   | 0.1 |
| % Pts Treated | 17.06 | 24.45 | 32.55 | 25.94 | 32.66 |  |

Note: “% Early Stopping” refers to early stopping due to excessive DLT.

* + 1. Analysis of Safety Endpoint

Frequency and severity of adverse events and tolerability of the regimen will be collected and summarized with descriptive statistics. The maximum grade for each type of toxicity will be recorded for each patient and frequency tables will be reviewed to determine toxicity patterns. All patients who received at least one dose of the triapine will be evaluable for toxicity and tolerability.

## Sample Size/Accrual Rate

**PLANNED ENROLLMENT REPORT**

| **DOMESTIC PLANNED ENROLLMENT REPORT (TREATMENT)** |
| --- |
| **Racial Categories** | **Ethnic Categories** | **Total** |
| **Not Hispanic or Latino** | **Hispanic or Latino** |
| **Female** | **Male** | **Female** | **Male** |
| American Indian/ Alaska Native | 0 | 0 | 0 | 0 | 0 |
| Asian | 1 | 1 | 0 | 0 | 2 |
| Native Hawaiian or Other Pacific Islander | 0 | 0 | 0 | 0 | 0 |
| Black or African American | 1 | 1 | 0 | 0 | 2 |
| White | 7 | 9 | 3 | 5 | 24 |
| More Than One Race |  |  |  |  | 0 |
| **Total** | 9 | 11 | 3 | 5 | 28 |

| **INTERNATIONAL (including Canadian participants) PLANNED ENROLLMENT REPORT (TREATMENT)** |
| --- |
| **Racial Categories** | **Ethnic Categories** | **Total** |
| **Not Hispanic or Latino** | **Hispanic or Latino** |
| **Female** | **Male** | **Female** | **Male** |
| American Indian/ Alaska Native | 0 | 0 | 0 | 0 | 0 |
| Asian | 0 | 0 | 0 | 0 | 0 |
| Native Hawaiian or Other Pacific Islander | 0 | 0 | 0 | 0 | 0 |
| Black or African American | 0 | 0 | 0 | 0 | 0 |
| White | 1 | 1 | 0 | 0 | 2 |
| More Than One Race | 0 | 0 | 0 | 0 | 0 |
| **Total** | 1 | 1 | 0 | 0 | 2 |

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## Stratification Factors

Not applicable.

## Analysis of Secondary Endpoints

Triapine in plasma and peri-tumoral dialysate will be quantified using a validated LC-MS/MS assay (Matsumoto *et al.,* 2017). Plasma PK parameters will be derived and compared to historical controls. Dialysate concentrations will be calculated at multiple time points. Exploratorily, exposure-response relationships will be evaluated.

Response rate will be categorized based on the Response Assessment in Neuro-Oncology (RANO 2.0) criteria, in which tumor response is based on clinical history, physical exam, and MRI imaging findings (Leao *et al.,* 2020). The total number of patients in each response category divided by the total number of evaluable patients determines response rate. An evaluable patient is defined as an eligible patient who received at least one dose of triapine. The response rates will be calculated with corresponding 95% exact Clopper-Pearson confidence intervals. Kaplan Meier curves will estimate PFS and OS. PFS is defined as time from study enrollment to date of either disease progression or death. OS is defined as time from study enrollment to date of death. The proportion of evaluable patients who initiated bevacizumab for symptom control during the study period will be calculated using descriptive statistics.

For tumor tissue biomarkers, the presence of genetic alterations, expression of RRM2, and other molecular gene expression signatures will be descriptive in relation to tumor response and clinical outcomes.

# ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of AEs (Section 10.1) and the characteristics of an observed AE (Sections 10.2 and 10.3) will determine whether the event requires expedited reporting via the CTEP Adverse Event Reporting System (CTEP-AERS) **in addition** to routine reporting.

## Comprehensive Adverse Events and Potential Risks List (CAEPR)

* + 1. CAEPRs for CTEP IND Agent
			1. CAEPR for Triapine

**Comprehensive Adverse Events and Potential Risks list (CAEPR)**

**for**

**Triapine® (NSC 663249)**

The Comprehensive Adverse Events and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified withboldand italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic\_applications/docs/aeguidelines.pdf for further clarification. *Frequency is provided based on 182 patients.* Below is the CAEPR for Triapine®.

**NOTE:** Report AEs on the SPEER **ONLY IF** they exceed the grade noted in parentheses next to the AE in the SPEER. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

**Version 2.6, March 19, 2019**[**1**](#_ENREF_1)

|  **Adverse Events with Possible**  **Relationship to Triapine®** **(CTCAE 5.0 Term)****[n= 182]** |  |  **Specific Protocol Exceptions to Expedited Reporting (SPEER)** |
| --- | --- | --- |
| **Likely (>20%)** | **Less Likely (<=20%)** | **Rare but Serious (<3%)** |  |
| BLOOD AND LYMPHATIC SYSTEM DISORDERS |  |  |
| Anemia |  |  |  | ***Anemia (Gr 3)*** |
|  | Febrile neutropenia |  |  | ***Febrile neutropenia (Gr 2)*** |
|  |  | Hemolysis |  | ***Hemolysis (Gr 3)*** |
|  | Methemoglobinemia |  |  | ***Methemoglobinemia (Gr 2)*** |
| CARDIAC DISORDERS |  |  |
|  | Cyanosis |  |  | ***Cyanosis (Gr 2)*** |
|  |  | Left ventricular systolic dysfunction |  |  |
| GASTROINTESTINAL DISORDERS |  |  |
|  | Colitis |  |  | ***Colitis (Gr 2)*** |
|  | Constipation |  |  |  |
|  | Diarrhea |  |  | ***Diarrhea (Gr 2)*** |
|  | Dyspepsia |  |  | ***Dyspepsia (Gr 2)*** |
|  | Mucositis oral |  |  | ***Mucositis oral (Gr 2)*** |
| Nausea |  |  |  | ***Nausea (Gr 2)*** |
| Vomiting |  |  |  | ***Vomiting (Gr 2)*** |
| GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS |  |  |
|  | Chills |  |  | ***Chills (Gr 2)*** |
| Fatigue |  |  |  | ***Fatigue (Gr 2)*** |
| Fever |  |  |  | ***Fever (Gr 2)*** |
|  | Injection site reaction |  |  | ***Injection site reaction (Gr 2)*** |
| INFECTIONS AND INFESTATIONS |  |  |
|  | Infection2 |  |  | ***Infection2 (Gr 3)*** |
| INVESTIGATIONS |  |  |
|  | Alanine aminotransferase increased |  |  | ***Alanine aminotransferase increased (Gr 2)*** |
|  | Alkaline phosphatase increased |  |  | ***Alkaline phosphatase increased (Gr 2)*** |
|  | Aspartate aminotransferase increased |  |  | ***Aspartate aminotransferase increased (Gr 2)*** |
|  | Blood bicarbonate decreased |  |  | ***Blood bicarbonate decreased (Gr 2)*** |
|  | Blood bilirubin increased |  |  | ***Blood bilirubin increased (Gr 2)*** |
|  | Creatinine increased |  |  | ***Creatinine increased (Gr 2)*** |
|  | Electrocardiogram QT corrected interval prolonged |  |  | ***Electrocardiogram QT corrected interval prolonged (Gr 2)*** |
|  | Investigations - Other (Elevated ST and T wave changes) |  |  | ***Investigations - Other (Elevated ST and T wave changes) (Gr 2)*** |
|  | Lipase increased |  |  | ***Lipase increased (Gr 2)*** |
|  | Lymphocyte count decreased |  |  |  |
| Neutrophil count decreased |  |  |  | ***Neutrophil count decreased (Gr 4)*** |
| Platelet count decreased |  |  |  | ***Platelet count decreased (Gr 4)*** |
|  | Weight loss |  |  | ***Weight loss (Gr 2)*** |
| White blood cell decreased |  |  |  | ***White blood cell decreased (Gr 4)*** |
| METABOLISM AND NUTRITION DISORDERS |  |  |
|  | Anorexia |  |  | ***Anorexia (Gr 2)*** |
|  | Dehydration |  |  | ***Dehydration (Gr 2)*** |
|  | Hypercalcemia |  |  | ***Hypercalcemia (Gr 2)*** |
|  | Hyperkalemia |  |  | ***Hyperkalemia (Gr 2)*** |
|  | Hypoalbuminemia |  |  | ***Hypoalbuminemia (Gr 2)*** |
|  | Hypokalemia |  |  | ***Hypokalemia (Gr 2)*** |
| MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS |  |  |
|  | Myalgia |  |  |  |
| NERVOUS SYSTEM DISORDERS |  |  |
|  | Dizziness |  |  |  |
|  | Dysgeusia |  |  | ***Dysgeusia (Gr 2)*** |
|  | Headache |  |  |  |
| RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS |  |  |
|  | Cough |  |  | ***Cough (Gr 2)*** |
|  | Dyspnea |  |  | ***Dyspnea (Gr 2)*** |
|  | Hypoxia |  |  | ***Hypoxia (Gr 3)*** |
|  |  | Pneumonitis |  |  |
| SKIN AND SUBCUTANEOUS TISSUE DISORDERS |  |  |
|  | Alopecia |  |  | ***Alopecia (Gr 2)*** |
|  | Rash maculo-papular |  |  | ***Rash maculo-papular (Gr 2)*** |
| VASCULAR DISORDERS |  |  |
|  | Flushing |  |  | ***Flushing (Gr 2)*** |
|  | Hypertension |  |  | ***Hypertension (Gr 2)*** |
|  | Hypotension |  |  | ***Hypotension (Gr 2)*** |

1This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

2Infection includes all 75 sites of infection under the INFECTIONS AND INFESTATIONS SOC.

**Adverse events reported on Triapine® trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that Triapine® caused the adverse event:**

**BLOOD AND LYMPHATIC SYSTEM DISORDERS** - Blood and lymphatic system disorders - Other (spleen disorder); Blood and lymphatic system disorders - Other (monocytosis); Disseminated intravascular coagulation; Hemolytic uremic syndrome; Leukocytosis; Thrombotic thrombocytopenic purpura

**CARDIAC DISORDERS** - Atrial fibrillation; Cardiac disorders - Other (cardiovascular edema); Cardiac disorders - Other (premature ventricular contraction); Myocardial infarction; Palpitations; Pericardial effusion; Restrictive cardiomyopathy; Sinus tachycardia; Ventricular tachycardia

**EAR AND LABYRINTH DISORDERS** - Ear and labyrinth disorders - Other (ear congestion); Ear and labyrinth disorders - Other (hyperacusis); Ear pain; Hearing impaired; Middle ear inflammation; Tinnitus; Vertigo

**EYE DISORDERS** - Dry eye; Watering eyes

**GASTROINTESTINAL DISORDERS** - Abdominal pain; Ascites; Dry mouth; Dysphagia; Esophagitis; Flatulence; Gastrointestinal disorders - Other (glossitis); Gastrointestinal disorders - Other (leukoplakia of the mouth); Gastrointestinal disorders - Other (mouth ulceration); Gastrointestinal disorders - Other (salivary hypersecretion); Gastrointestinal disorders - Other (steatorrhea); Gastrointestinal disorders - Other (stool discoloration); Gastrointestinal disorders - Other (tongue discoloration); Hemorrhoids; Ileus; Oral hemorrhage; Pancreatitis; Rectal hemorrhage; Small intestinal obstruction

**GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS** - Death NOS; Edema face; Edema limbs; Flu like symptoms; General disorders and administration site conditions - Other (extravasation); Malaise; Non-cardiac chest pain; Pain; Sudden death NOS

**HEPATOBILIARY DISORDERS** - Hepatobiliary disorders - Other (hepatomegaly); Hepatobiliary disorders - Other (jaundice); Hepatobiliary disorders - Other (liver tenderness)

**IMMUNE SYSTEM DISORDERS** - Allergic reaction; Anaphylaxis; Cytokine release syndrome

**INJURY, POISONING AND PROCEDURAL COMPLICATIONS** - Infusion related reaction

**INVESTIGATIONS** - Activated partial thromboplastin time prolonged; Blood lactate dehydrogenase increased; CPK increased; Cholesterol high; GGT increased; INR increased; Investigations - Other (BUN increased); Investigations - Other (C-reactive protein increased); Investigations - Other (NPN increased); Investigations - Other (PT decreased); Investigations - Other (sedimentation rate increased); Serum amylase increased; Weight gain

**METABOLISM AND NUTRITION DISORDERS** - Acidosis; Alkalosis; Hyperglycemia; Hypernatremia; Hyperuricemia; Hypocalcemia; Hypoglycemia; Hypomagnesemia; Hyponatremia; Hypophosphatemia; Iron overload; Metabolism and nutrition disorders - Other (hypoproteinemia); Tumor lysis syndrome

**MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS** - Arthralgia; Arthritis; Back pain; Bone pain; Chest wall pain; Generalized muscle weakness; Musculoskeletal and connective tissue disorder - Other (hypertonia); Musculoskeletal and connective tissue disorder - Other (leg cramps); Musculoskeletal and connective tissue disorder - Other (myoglobin); Musculoskeletal and connective tissue disorder - Other (twitching); Pain in extremity

**NERVOUS SYSTEM DISORDERS** - Amnesia; Cognitive disturbance; Depressed level of consciousness; Dysesthesia; Intracranial hemorrhage; Ischemia cerebrovascular; Lethargy; Nervous system disorders - Other (cerebellar toxicity); Nervous system disorders - Other (reflexes decreased); Paresthesia; Peripheral motor neuropathy; Peripheral sensory neuropathy; Seizure; Somnolence; Syncope; Tremor

**PSYCHIATRIC DISORDERS** - Anxiety; Confusion; Delayed orgasm; Delirium; Depression; Insomnia; Personality change

**RENAL AND URINARY DISORDERS** - Acute kidney injury; Cystitis noninfective; Hematuria; Urinary frequency; Urinary tract pain; Urine discoloration

**REPRODUCTIVE SYSTEM AND BREAST DISORDERS** - Erectile dysfunction; Genital edema; Vaginal hemorrhage

**RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS** - Adult respiratory distress syndrome; Apnea; Epistaxis; Hiccups; Laryngospasm; Pleural effusion; Voice alteration

**SKIN AND SUBCUTANEOUS TISSUE DISORDERS** - Dry skin; Erythema multiforme; Hirsutism; Hyperhidrosis; Photosensitivity; Pruritus; Skin and subcutaneous tissue disorders - Other (Skin nodule); Skin ulceration; Stevens-Johnson syndrome

**VASCULAR DISORDERS** - Hematoma; Phlebitis; Thromboembolic event; Vascular disorders - Other (pallor); Vascular disorders - Other (vasodilation)

**Note**: Triapine® in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

## Adverse Event Characteristics

* **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP website <http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm>.
* **For expedited reporting purposes only:**
* AEs for the agent that are ***bold and italicized*** in the CAEPR (*i.e.*, those listed in the SPEER column, Section 10.1) should be reported through CTEP-AERS only if the grade is above the grade provided in the SPEER.
* **Attribution** of the AE:
	+ Definite – The AE *is clearly related* to the study treatment.
	+ Probable – The AE *is likely related* to the study treatment.
	+ Possible – The AE *may be related* to the study treatment.
	+ Unlikely – The AE *is doubtfully related* to the study treatment.
	+ Unrelated – The AE *is clearly NOT related* to the study treatment.

## Expedited Adverse Event Reporting

* + 1. Rave-CTEP-AERS Integration

The Rave Cancer Therapy Evaluation Program Adverse Event Reporting System (CTEP-AERS) integration enables evaluation of Adverse Events (AEs) entered in Rave to determine whether they require expedited reporting and facilitates entry in CTEP-AERS for those AEs requiring expedited reporting. Sites must initiate all AEs for this study in Medidata Rave.

Treatment-emergent AEs: All AEs that occur after start of treatment are collected in Medidata Rave using the Adverse Event form, which is available for entry at each treatment course or reporting period and is used to collect AEs that start during the period or persist from the previous reporting period. AEs that occur 30 days after the last administration of the investigational agent/intervention are collected using the Late Adverse Event form.

Prior to sending AEs through the rules evaluation process, site staff should verify the following on the Adverse Event form in Rave:

* The reporting period (course/cycle) is correct, and
* AEs are recorded and complete (no missing fields) and the form is query free.

The CRA reports AEs in Rave at the time the Investigator learns of the event. If the CRA modifies an AE, it must be re-submitted for rules evaluation.

Upon completion of AE entry in Medidata Rave, the CRA submits the AE for rules evaluation by completing the Expedited Reporting Evaluation form (*i.e.*, checking the box *Send All AEs for Evaluation* and save the form). Both NCI and protocol-specific reporting rules evaluate the AEs submitted for expedited reporting. A report is initiated in CTEP-AERS using information entered in Medidata Rave for AEs that meet reporting requirements. The CRA completes the report by accessing CTEP-AERS via a direct link on the Medidata Rave Expedited Reporting Evaluation form. Contact the CTSU Help Desk at 1-888-823-5923 or by email at ctsucontact@westat.com if you have any issues submitting an expedited report in CTEP-AERS.

In the rare occurrence that internet connectivity is lost, a 24-hour notification is to be made to CTEP by telephone at 301-897-7497. Once internet connectivity is restored, the 24-hour notification that was phoned in must be entered immediately into CTEP-AERS using the direct link from Medidata Rave.

Additional information about the CTEP-AERS integration is available on the CTSU members’ website:

* Study specific documents: *Protocols > Documents> Protocol Related Documents> Adverse Event Reporting*, and
* Additional resources: *Resources > CTSU Operations Information> User Guides & Help Topics*.

NCI requirements for SAE reporting are available on the CTEP website:

* NCI Guidelines for Investigators: Adverse Event Reporting Requirements is available at <https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf>.
	+ 1. Distribution of Adverse Event Reports

CTEP-AERS is programmed for automatic electronic distribution of reports to the following individuals: Principal Investigator and Adverse Event Coordinator(s) (if applicable) of the Corresponding Organization or Lead Organization, the local treating physician, and the Reporter and Submitter. CTEP-AERS provides a copy feature for other e-mail recipients.

* + 1. Expedited Reporting Guidelines

Use the NCI protocol number and the protocol-specific patient ID assigned during trial registration on all reports.

**Note: A death on study requires both routine and expedited reporting, regardless of causality as long as the death occurred within 30 days after the last administration of the investigational agent. Attribution to treatment or other cause must be provided.**

Death due to progressive disease should be reported as **Grade 5 “Disease progression”**in the system organ class (SOC) “General disorders and administration site conditions.” Evidence that the death was a manifestation of underlying disease (*e.g.*, radiological changes suggesting tumor growth or progression: clinical deterioration associated with a disease process) should be submitted.

**Phase 1 and Early Phase 2 Studies: Expedited Reporting Requirements for Adverse Events that Occur on Studies under an IND/IDE within 30 Days of the Last Administration of the Investigational Agent/Intervention 1, 2**

|  |
| --- |
| **FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)****NOTE:** Investigators **MUST** immediately report to the sponsor (NCI) **ANY** SAEs, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64).An AE is considered serious if it results in **ANY** of the following outcomes:1. Death
2. A life-threatening AE
3. An AE that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours.
4. A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5. A congenital anomaly/birth defect.
6. Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).
 |
| **ALL SAEs** that meet the above criteria **MUST** be immediately reported to the NCI via CTEP-AERS within the timeframes detailed in the table below. |
| **Grade 1-2 Timeframes** | **Grade 3-5 Timeframes** |
| 24-Hour notification, 10 Calendar Days | 24-Hour notification, 5 Calendar Days |
| **NOTE:** Protocol-specific exceptions to expedited reporting of SAEs are found in the Specific Protocol Exceptions to Expedited Reporting (SPEER) portion of the CAEPR.**Expedited AE reporting timeframes are defined as:*** “24-Hour notification, 5 Calendar Days” - The SAE must initially be reported via CTEP-AERS within 24 hours of learning of the SAE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.
* “24-Hour notification, 10 Calendar Days” - The SAE must initially be reported via CTEP-AERS within 24 hours of learning of the SAE, followed by a complete expedited report within 10 calendar days of the initial 24-hour report.
 |
| 1SAEs that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:**Expedited 24-Hour notifications are required for all SAEs followed by a complete report*** Within 5 calendar days for Grade 3-5 SAEs
* Within 10 calendar days for Grade 1-2 SAEs

2For studies using nuclear medicine or molecular imaging IND agents (NM, SPECT, or PET), the SAE reporting period is limited to 10 radioactive half-lives, rounded UP to the nearest whole day, after the agent/intervention was last administered. Footnote “1” above applies after this reporting period.Effective Date: August 30, 2024 |

## Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions. **AEs reported expeditiously through CTEP-AERS must also be reported in routine study data submissions.**

Adverse event data collection and reporting, which are required as part of every clinical trial, are done to ensure the safety of patients enrolled in the studies as well as those who will enroll in future studies using similar agents. AEs are reported in a routine manner at scheduled times during the trial using Medidata Rave. For this trial the Adverse Event CRF is used for routine AE reporting in Rave.

## Pregnancy

Although not an adverse event in and of itself, pregnancy as well as its outcome must be documented via **CTEP-AERS**. In addition, the ***Pregnancy Information Form*** included within the NCI Guidelines for Adverse Event Reporting Requirements must be completed and submitted to CTEP. Any pregnancy occurring in a patient or patient’s partner from the time of consent to 90 days after the last dose of study drug must be reported and then followed for outcome. Newborn infants should be followed until 30 days old. Please see the “NCI Guidelines for Investigators: Adverse Event Reporting Requirements for DCTD (CTEP and CIP) and DCP INDs and IDEs” (at <http://ctep.cancer.gov/protocolDevelopment/adverse_effects.htm>) for more details on how to report pregnancy and its outcome to CTEP.

## Secondary Malignancy

A *secondary malignancy* is a cancer caused by treatment for a previous malignancy (*e.g.*, treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.

CTEP requires all secondary malignancies that occur following treatment with an agent under an NCI IND/IDE be reported expeditiously via CTEP-AERS. Three options are available to describe the event:

* Leukemia secondary to oncology chemotherapy (*e.g.*, acute myelocytic leukemia [AML])
* Myelodysplastic syndrome (MDS)
* Treatment-related secondary malignancy

Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in each protocol.

## Second Malignancy

A *second malignancy* is one unrelated to the treatment of a prior malignancy (and is **NOT** a metastasis from the initial malignancy). Second malignancies require **ONLY** routine AE reporting unless otherwise specified.

# STUDY CALENDAR

Baseline evaluations are to be conducted within 4 weeks prior to start of protocol therapy. Scans and x-rays must be done <4 weeks prior to the start of therapy. In the event that the patient’s condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next therapy exposure.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | Pre-Study | Wk1 | Wk2 | Wk3 | Wk4 | Wk5 | Wk6 | Wk7 | Wk8 | Wk9 | Wk10 | Wk11 | Wk12 | Off Studya |
| Triapine |  | A | A |  |  |  |  |  |  |  |  |  |  |  |
| Radiation Therapy |  | B | B |  |  |  |  |  |  |  |  |  |  |  |
| Informed consent | X |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Demographics | X |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Medical history | X |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Concurrent meds | X | X---------------------------------------------------------------------------------------------X |  |
| Physical exam | X | X | X |  | X |  |  |  |  |  |  |  | X | X |
| Vital signs | X | X | X |  | X |  |  |  |  |  |  |  | X | X |
| Height | X |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Weight | X | X |  |  | X |  |  |  |  |  |  |  | X | X |
| Performance statusb | X | X | X |  | X |  |  |  |  |  |  |  | X | X |
| CBC w/diff, plts | X | X | X |  | X |  |  |  |  |  |  |  |  |  |
| Serum chemistryc | X | X | X |  | X |  |  |  |  |  |  |  |  |  |
| Adverse event evaluation |  | X---------------------------------------------------------------------------------------------X | X |
| Tumor measurements | X | Tumor measurements are repeated every 12 weeks. Documentation (radiologic) must be provided for patients removed from study for progressive disease. | X |
| Radiologic evaluation | X | Radiologic measurements should be performed at week 4 and every 12 weeks thereafter for up to 2 years. | X |
| Pregnancy testd | X |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Archival Tissuee | X |  |  |  |  |  |  |  |  |  |  |  |  |  |
| CSF in microvialf | X |  |  |  |  |  |  |  |  |  |  |  |  |  |
| Blood in purple-top EDTA Tubes | Xf | Xg | Xg |  |  |  |  |  |  |  |  |  |  |  |
| Blood in K2 EDTA Tubes | X |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A:Triapine: Dose as assigned; administered within 2 hours prior to radiation therapy. No dose will be administered during non-radiation treatment days .B: Radiation Therapy: 3.5 Gy per fraction for 10 fractions, given once daily during business days only.a: Off-study evaluation.b: Performance status will be assessed at baseline, during radiation treatments, and scheduled follow-ups.c: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.d: Pregnancy test for women of childbearing potential.e: Archival tumor tissue from initial diagnosis or recurrence; no re-biopsies are planned or allowed. For patients scheduled for surgery prior to enrollment onto study, surgical tissue may be used instead of archival tissue. Optional. f: Applicable for only select patients undergoing resection for recurrent tumor treated at City of Hope who have agreed to intracranial microdialysis sub-study of triapine. Blood and CSF collection will be at least 24 hours after surgery but within 48 hours after surgery. Further details are found in [Section 5.5.2.3](#Section_5_5_2_3). Afterwards, these patients will be provided with the opportunity to enroll on to the main trial.g: Blood collected into purple-top EDTA tube and processed to plasma. Collection times are: Week 1, Day 1, 1-3 hours post-dose, Week 1 Day 2, pre-dose, 0.5, 1, 1.5, 2, 3, 4, 6, and 8 hours post-dose. Alternative radiation treatment days are acceptable in place of Week 1 Day 2. |

# MEASUREMENT OF EFFECT

Although the clinical benefit of this drug has not yet been established, the intent of offering this treatment is to provide a possible therapeutic benefit, and thus the patient will be carefully monitored for tumor response and symptom relief in addition to safety and tolerability. Patients with measurable disease will be assessed by standard criteria. For the purposes of this study, patients should be re-evaluated every 12weeks. In addition to a baseline scan, confirmatory scans will also be obtained 4weeks following initial documentation of an objective response.

## Antitumor Effect – Solid Tumors

For the purposes of this study, patients should be re-evaluated for response every 12weeks. In addition to a baseline scan, confirmatory scans should also be obtained 4 weeks following initial documentation of objective response.

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [*Eur J Ca* 45:228-247, 2009]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

* + 1. Definitions

Evaluable for Toxicity. All patients will be evaluable for toxicity from the time of their first treatment with triapine.

Evaluable for Objective Response. Only those patients who have measurable disease present at baseline, have at least 5 fractions of radiation therapy, 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 treatment will also be considered evaluable.)

Evaluable Non-Target Disease Response. Patients who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least 5 fractions of radiation therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

* + 1. Disease Parameters

Measurable Disease. Measurable lesions are defined as contrast-enhancing or non-contrast enhancing lesions with clearly defined margins by MRI scan, with both perpendicular diameters on a single slice in at least one dimension (longest diameter to be recorded) as at least 10mm (1cm) visible on at least two or more slices with slice thickness < 5mm. In the event the MRI is performed with thicker slice thickness, the size of measurable lesion for both perpendicular measurements should be two times the slice thickness and interslice gap. Any cystic or surgical cavity should not be measured in determining therapeutic response. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Note: Tumor lesions that are situated in a previously irradiated area might be considered measurable. Measurement of tumor around a previously irradiated area remains challenging. Solid, contrast-enhancing lesions measuring > 10 x 10mm in diameter can be considered measurable.

Non-Measurable Disease. All other lesions, including small lesions (longest diameter <10 mm [<1 cm], are considered non-measurable disease. Patients without measurable disease, such as those who have undergone a gross total resection, cannot exhibit a response to treatment; therefore, can only achieve stable disease as their best radiologic outcome assuming treatment is started before there is radiologic evidence of new tumor growth. Therefore, only patients with measurable disease can be included in the assessment of overall response rate, while patients without measurable disease may be included in assessments for other outcomes such as toxicity or time-to-event endpoints.

Target Lesions. All measurable lesions up to a maximum of 2 lesions and no more than 3 lesions (enhancing or non-enhancing) should be identified as **target lesions** and recorded and measured at baseline. The enhancing lesion(s) can be within a non-enhancing tumor. A sum of the products of the perpendicular for all target lesions will be calculated and reported as the baseline. Generally, the largest enlarging lesion(s) should be selected. For patients with multiple lesions, those that are increasing in size should be selected as target lesions, regardless of their relative size.

Non-Target Lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 3 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

* + 1. Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Conventional CT and MRI. This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm (0.5 cm) or less. If CT scans have slice thickness greater than 5 mm (0.5 cm), the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (*e.g.* for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used, and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

Cytology, Histology. These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (*e.g.*, residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

* + 1. Response Criteria
			1. Evaluation of Target and Non-Target Lesions

Complete Response (CR): Disappearance of all measurable, non-measurable, and non-target disease for at least 4 weeks, no new lesions, patients are off corticosteroids (or on physiologic replacement doses only), and patients are clinically stable or improving.

Partial Response (PR): At least a 50% decrease in the sum of products of perpendicular diameters (or ≥ 65% decrease in total volume) of measurable target lesions, taking as reference the baseline sum diameters. Sustained for at least 4 weeks. There are no new lesions, no progression of non-measurable enhancing or non-target lesions, patients are off corticosteroids (or on physiologic replacement doses only), and patients are clinically stable or improving.

Progressive Disease (PD): At least a 25% increase in the sum of products of perpendicular diameters of all measurable target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition, a relative increase of ≥40% in total volume of target lesions is considered PD. The appearance of one or more new lesions, leptomeningeal disease, clear progression of non-measurable lesions, unequivocal progression of existing non-target lesions, definite clinical deterioration not attributable to decrease in corticosteroid dose or other causes apart from the tumor, or failure to return for evaluation as a result of death or deteriorating condition is also considered disease progression (unless caused by documented non-related disorders).

If confirmation scans are required for disease progression, then at least 2 sequential scans separated by ≥ 4 weeks both exhibiting ≥ 25% increase in sum of products of the diameters of target lesions (or ≥ 40% increase in total volume) compared to the most recent previous scan will be required. If the second scan at least 4 weeks later exhibits SD or PR/CR, the previous scan showing preliminary PD is noted as pseudo-progression.

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum diameters while on study.

* + - 1. Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria. Please refer to [Appendix E](#_APPENDIX_E_RANO).

* + 1. 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.

* + 1. Progression-Free Survival (PFS)

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

* + 1. Overall Survival (OS)

OS is defined as the duration of time from start of treatment to time of death.

# STUDY OVERSIGHT AND DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 10 (Adverse Events: List and Reporting Requirements).

## Study Oversight

This protocol is monitored at several levels, as described in this section. The Protocol Principal Investigator is responsible for monitoring the conduct and progress of the clinical trial, including the ongoing review of accrual, patient-specific clinical and laboratory data, and routine and serious adverse events; reporting of expedited adverse events; and accumulation of reported adverse events from other trials testing the same drug(s). The Protocol Principal Investigator and statistician have access to the data at all times through the CTMS web-based reporting portal.

For the Phase 1 portion of this study, all decisions regarding dose escalation/expansion/de-escalation require sign-off by the Protocol Principal Investigator through the CTMS/IWRS. In addition, for the Phase 1 portion, the Protocol Principal Investigator will have at least monthly conference calls with the Study Investigators [and, if needed, the CTEP Medical Officer(s)] to review accrual, progress, and adverse events and unanticipated problems.

All Study Investigators at participating sites who register/enroll patients on a given protocol are responsible for timely submission of data via Medidata Rave and timely reporting of adverse events for that particular study. This includes timely review of data collected on the electronic CRFs submitted via Medidata Rave.

All studies are also reviewed in accordance with the enrolling institution’s data safety monitoring plan.

## Data Reporting

Medidata Rave is the clinical data management system being used for data collection for this trial/study. Access to the trial in Rave is controlled through the CTEP-IAM system and role assignments.

Requirements to access Rave via iMedidata:

* Active CTEP registration with the credentials necessary to access secure NCI/CTSU IT systems; and
* Assigned a Rave role on the LPO or PO roster at the enrolling site of: Rave CRA, Rave Read Only, Rave CRA (LabAdmin), Rave SLA, or Rave Investigator.
* Rave role requirements:
	+ - Rave CRA or Rave CRA (Lab Admin) role must have a minimum of an Associate Plus (AP) registration type,
		- Rave Investigator role must be registered as a Non-Physician Investigator (NPIVR) or Investigator (IVR), and
		- Rave Read Only or Rave SLA role must have at a minimum an Associate (A) registration type.
	+ Refer to <https://ctep.cancer.gov/investigatorResources/default.htm> for registration types and documentation required.
* DTL Rave CRA task assignment requirements (for write access):
* Corresponding role (Rave CRA or Rave CRA (Lab Admin)) on the site roster; and
* Completion of the Theradex Specimen Tracking System (STS) Training course in CLASS (see Section 4.2.3 – Delegation of Tasks Log (DTL)).

Protocol Specific Requirements For Rave Access

* + - Specimen Tracking System Training Requirement for full read/write Rave access:
		- All site staff assigned the Rave CRA task on the DTL must complete the online specimen tracking training, which is administered via the Compliance, Learning, and SOP Solutions (CLASS) system.
		- Completion of the training will be automatically communicated to the CTSU Regulatory application and to Medidata Rave, and the individual will receive an invitation to 10699 in Rave. *There is no need to submit a training completion certificate to* *the CTSU through the Regulatory Submission Portal.*
		- The training is a one-time only requirement per individual. If an individual has previously completed the training for another ETCTN study (either within CLASS, or via the procedure in place prior to CLASS), the training does not need to be completed again. However, new versions of the Specimen Tracking System training course may require new training.
		- For questions about the training content or the tracking system itself, please contact STS Support at Theradex (STS.Support@theradex.com).
		- For questions or concerns about **accessing the training in CLASS**, please contact the CLASS Help Desk CLASSHelpDesk@westat.com.

Upon initial site registration approval for the study in the Regulatory application, all persons with Rave roles assigned on the appropriate roster will be sent a study invitation email from iMedidata.  No action will be required; each study invitation will be automatically accepted and study access in Rave will be automatically granted. Site staff will not be able to access the study in Rave until all required Medidata and study specific trainings are completed.  Trainings will be in the form of electronic learnings (eLearnings) and can be accessed by clicking on the eLearning link in the *Tasks* pane located in the upper right corner of the iMedidata screen. If an eLearning is required for a study and has not yet been taken, the link to the eLearning will appear under the study name in the *Studies* pane located in the center of the iMedidata screen; once the successful completion of the eLearning has been recorded, access to the study in Rave will be granted, and a *Rave EDC* link will replace the eLearning link under the study name.

Action will be required by site staff (to activate their account) who have not previously activated their iMedidata/Rave account at the time of initial site registration approval for the study in the Regulatory application. Account activation instructions are located on the CTSU website in the Data Management section under the Data Management Help Topics > Rave Resources > [Medidata Account Activation and Study Invitation](https://www.ctsu.org/master/simplepage.aspx?ckey=HELP-DQP#RaveResources) (to activate your iMedidata account). Additional information on iMedidata/Rave is available on the CTSU members’ website in the Data Management [Rave Resources](https://www.ctsu.org/master/simplepage.aspx?ckey=HELP-DQP#RaveResources) section or by contacting the CTSU Help Desk at 1-888-823-5923 or by email at ctsucontact@westat.com.

* + 1. Method

*For studies assigned for* ***CTMS Comprehensive*** *Monitoring:*

This study will be monitored by the Clinical Trials Monitoring Service (CTMS). Data will be submitted to CTMS at least once every two weeks via Medidata Rave (or other modality if approved by CTEP). Information on CTMS reporting is available at <http://www.theradex.com/clinicalTechnologies/?National-Cancer-Institute-NCI-11>. On-site audits will be conducted three times annually (one annual site visit and two data audits). For CTMS monitored studies, after users have activated their accounts, please contact the Theradex Help Desk at (609) 619-7862 or by email at CTMSSupport@theradex.com for additional support with Rave and completion of CRFs.

*For studies assigned for* ***CTMS Routine*** *Monitoring:*

This study will be monitored by the Clinical Trials Monitoring Service (CTMS). Data will be submitted to CTMS at least once every two weeks via Medidata Rave (or other modality if approved by CTEP). Information on CTMS reporting is available at: <http://www.theradex.com/clinicalTechnologies/?National-Cancer-Institute-NCI-11>. On-site audits will be conducted on an 18-36 month basis. More frequent audits may be conducted if warranted by accrual or due to concerns regarding data quality or timely submission. For CTMS monitored studies, after users have activated their accounts, please contact the Theradex Help Desk at (609) 619-7862 or by email at CTMSSupport@theradex.com for additional support with Rave and completion of CRFs.

* + 1. Responsibility for Data Submission

For ETCTN trials, it is the responsibility of the PI(s) at the site to ensure that all investigators at the ETCTN Sites understand the procedures for data submission for each ETCTN protocol and that protocol specified data are submitted accurately and in a timely manner to the CTMS via the electronic data capture system, Medidata Rave.

Data are to be submitted via Medidata Rave to CTMS on a real-time basis, but no less than once every 2 weeks. The timeliness of data submissions and timeliness in resolving data queries will be tracked by CTMS. Metrics for timeliness will be followed and assessed on a quarterly basis. For the purpose of Institutional Performance Monitoring, data will be considered delinquent if it is greater than 4 weeks past due.

Data from Medidata Rave and CTEP-AERS is reviewed by the CTMS on an ongoing basis as data is received. Queries will be issued by CTMS directly within Rave. The queries will appear on the Task Summary Tab within Rave for the CRA at the ETCTN to resolve. Monthly web-based reports are posted for review by the Drug Monitors in the IDB, CTEP. Onsite audits will be conducted by the CTMS to ensure compliance with regulatory requirements, GCP, and NCI policies and procedures with the overarching goal of ensuring the integrity of data generated from NCI-sponsored clinical trials, as described in the ETCTN Program Guidelines, which may be found on the CTEP (<http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm>) and CTSU websites.

CTMS will utilize a core set of eCRFs that are Cancer Data Standards Registry and Repository (caDSR) compliant (<https://datascience.cancer.gov/resources/metadata>). Customized eCRFs will be included when appropriate to meet unique study requirements. The PI is encouraged to review the eCRFs, working closely with CTMS to ensure prospectively that all required items are appropriately captured in the eCRFs prior to study activation. CTMS will prepare the eCRFs with built-in edit checks to the extent possible to promote data integrity.

CDUS data submissions for ETCTN trials activated after March 1, 2014, will be carried out by the CTMS contractor, Theradex. CDUS submissions are performed by Theradex on a monthly basis. The trial’s lead institution is responsible for timely submission to CTMS via Rave, as above.

Further information on data submission procedures can be found in the ETCTN Program Guidelines (<http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm>).

## Data Quality Portal

The Data Quality Portal (DQP) provides a central location for site staff to manage unanswered queries and form delinquencies, monitor data quality and timeliness, generate reports, and review metrics.

The DQP is located on the CTSU members’ website under Data Management. The Rave Home section displays a table providing summary counts of Total Delinquencies and Total Queries. DQP Queries, DQP Delinquent Forms, DQP Form Status and the DQP Reports modules are available to access details and reports of unanswered queries, delinquent forms, forms with current status, and timeliness reports. Site staff should review the DQP modules on a regular basis to manage specified queries and delinquent forms.

The DQP is accessible by site staff who are rostered to a site and have access to the CTSU website.  Staff who have Rave study access can access the Rave study data via direct links available on the DQP modules.

CTSU Delinquency Notification emails are sent to primary contacts at sites twice a month. These notifications serve as alerts that queries and/or delinquent forms require site review, providing a summary count of queries and delinquent forms for each Rave study that a site is participating in. Additional site staff can subscribe and unsubscribe to these notifications using the CTSU Report and Information Subscription Portal on the CTSU members’ website.

To learn more about DQP use and access, click on the Help Topics button displayed on the Rave Home, DQP Queries, DQP Delinquent Forms, DQP Form Status, and DQP Reports modules.

## CTEP Multicenter Guidelines

Not applicable.

## Collaborative Agreements Language

The agent(s) supplied by CTEP, DCTD, NCI used in this protocol is/are provided to the NCI under a Collaborative Agreement (CRADA, CTA, CSA) between the Pharmaceutical Company(ies) (hereinafter referred to as “Collaborator(s)”) and the NCI Division of Cancer Treatment and Diagnosis. Therefore, the following obligations/guidelines, in addition to the provisions in the “Intellectual Property Option to Collaborator”

(<http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm>) contained within the terms of award, apply to the use of the Agent(s) in this study:

1. Agent(s) may not be used for any purpose outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing Agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a copy of this protocol is requested by a patient or patient’s family member participating on the study, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from: <http://ctep.cancer.gov>.

2. For a clinical protocol where there is an investigational Agent used in combination with (an)other Agent(s), each the subject of different Collaborative Agreements, the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-Party Data”):

a. NCI will provide all Collaborators with prior written notice regarding the existence and nature of any agreements governing their collaboration with NCI, the design of the proposed combination protocol, and the existence of any obligations that would tend to restrict NCI's participation in the proposed combination protocol.

b. Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator solely to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval or commercialize its own Agent.

c. Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own Agent.

3. Clinical Trial Data and Results and Raw Data developed under a Collaborative Agreement will be made available to Collaborator(s), the NCI, and the FDA, as appropriate and unless additional disclosure is required by law or court order as described in the IP Option to Collaborator (<http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm>). Additionally, all Clinical Data and Results and Raw Data will be collected, used and disclosed consistent with all applicable federal statutes and regulations for the protection of human subjects, including, if applicable, the *Standards for Privacy of Individually Identifiable Health Information* set forth in 45 C.F.R. Part 164.

4. When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators (Group Chair for Cooperative Group studies, or PI for other studies) of Collaborator's wish to contact them.

5. Any data provided to Collaborator(s) for Phase 3 studies must be in accordance with the guidelines and policies of the responsible Data Monitoring Committee (DMC), if there is a DMC for this clinical trial.

6. Any manuscripts reporting the results of this clinical trial must be provided to CTEP by the Group office for Cooperative Group studies or by the principal investigator for non-Cooperative Group studies for immediate delivery to Collaborator(s) for advisory review and comment prior to submission for publication. Collaborator(s) will have 30 days from the date of receipt for review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborator’s confidential and proprietary data, in addition to Collaborator(s)’s intellectual property rights, are protected. Copies of abstracts must be provided to CTEP for forwarding to Collaborator(s) for courtesy review as soon as possible and preferably at least three (3) days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be forwarded to CTEP prior to release. Copies of any manuscript, abstract and/or press release/ media presentation should be sent to:

Email: ncicteppubs@mail.nih.gov

The Regulatory Affairs Branch will then distribute them to Collaborator(s). No publication, manuscript or other form of public disclosure shall contain any of Collaborator’s confidential/ proprietary information.

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# APPENDIX A PERFORMANCE STATUS CRITERIA

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

# APPENDIX B FORMULA TO ESTIMATE RENAL FUNCTION USING SERUM CREATININE

Formulas to estimate renal function using serum creatinine provided by the NCI’s Investigational Drug Steering Committee (IDSC) Pharmacological Task Force in table below.

|  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
| 1. Estimated glomerular filtration rate (eGFR) using the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) (Levey *et al.*, 2009).

Formulae:

|  |  |  |
| --- | --- | --- |
| **Race and Sex** | **Serum Creatinine (SCr), *µmol/L (mg/dL)*** | **Equation** |
| **Black** |  |  |
| Female | ≤62 (≤0.7) | GFR = 166 × (SCr/0.7)−0.329 × (0.993)Age |
|  | >62 (>0.7) | GFR = 166 × (SCr/0.7)−1.209 × (0.993)Age |
| Male | ≤80 (≤0.9) | GFR = 163 × (SCr/0.9)−0.411 × (0.993)Age |
|  | >80 (>0.9) | GFR = 163 × (SCr/0.9)−1.209 × (0.993)Age |
|  |  |  |
| **White or other** |  |  |
| Female | ≤62 (≤0.7) | GFR = 144 × (SCr/0.7)−0.329 × (0.993)Age |
|  | >62 (>0.7) | GFR = 144 × (SCr/0.7)−1.209 × (0.993)Age |
| Male | ≤80 (≤0.9) | GFR = 141 × (SCr/0.9)−0.411 × (0.993)Age |
|  | >80 (>0.9) | GFR = 141 × (SCr/0.9)−1.209 × (0.993)Age |

SCr in mg/dL; Output is in mL/min/1.73 m2 and needs no further conversions. |
| 1. eGFR using the Modification of Diet in Renal Disease (MDRD) Study (Levey *et al*., 2006).

175 x SCr–1.154 × age–0.203 × 0.742 (if female) × 1.212 (if black)Output is in mL/min/1.73 m2 and needs no further conversions. |
| 1. Estimated creatinine clearance (ClCr) by the Cockcroft-Gault (C-G) equation (Cockcroft and Gault, 1976).

Followed by conversion to a value normalized to 1.73 m2 with the patient’s body surface area (BSA). |

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3. Cockcroft, D.W. and M.H. Gault. (1976). Prediction of creatinine clearance from serum creatinine. *Nephron*. 16:31-41.

# APPENDIX C PATIENT CLINICAL TRIAL WALLET CARD

****

|  |
| --- |
|  |
| **cliniCal trial wallet card** |
| **Show this card to all of your healthcare providers and keep it with you in case you go to the emergency room.** |
| **Patient Name:**       |
| **Diagnosis:**       |
| **Study Doctor:**       |
| **Study Doctor Phone #:**       |
| **NCI Trial #:**       |
| **Study Drug:** Triapine |
|
| **For more information:** 1-800-4-CANCER |
| cancer.gov | clinicaltrials.gov |

# APPENDIX D PATIENT MEDICATION DIARY

The medication diary is located on the next page.

**PATIENT’S MEDICATION DIARY – Triapine**

**Today’s Date \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_** **Cycle \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ CTEP-assigned Protocol #** **10699**

**Patient Study ID \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Patient Initials \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Local Protocol #**

INSTRUCTIONS TO THE PATIENT:

1. Complete one form for each cycle. Each cycle is 28 days.
2. You will take your assigned number of triapine capsules each day **in the morning**, within 2 hours prior to radiation therapy.
3. You need to fast (except for water and medication) for 2 hours prior to taking the capsule and for 1 hour after ingesting the capsule.
4. **Keep the capsules intact, they must not be open, broken, chewed, or crushed**.
5. Record the date, the number of capsules you took, and when you took them.
6. If you have any comments or notice any side effects, please record them in the Comments column for that day.
7. Please return this form and your bottle(s) ofcapsules to your physician when you go for your next appointment.
8. Notify your doctor at the first sign of poorly formed or loose stools, or an increased frequency of bowel movements. Loperamide should be kept on hand and should be taken as recommended by your doctor.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Day** | **Date** | **Time of dose** | **# of capsules taken** | **Comments** |
| 1 |  |  |  |  |
| 2 |  |  |  |  |
| 3 |  |  |  |  |
| 4 |  |  |  |  |
| 5 |  |  |  |  |
| 6 | XX | XX | XX | Do not take triapine on this day |
| 7 | XX | XX | XX | Do not take triapine on this day |
| 8 |  |  |  |  |
| 9 |  |  |  |  |
| 10 |  |  |  |  |
| 11 |  |  |  |  |
| 12 |  |  |  |  |
| 13 | XX | XX | XX | Do not take triapine on this day |
| 14 | XX | XX | XX | Do not take triapine on this day |
| 15 | XX | XX | XX | Do not take triapine on this day |
| 16 | XX | XX | XX | Do not take triapine on this day |
| 17 | XX | XX | XX | Do not take triapine on this day |
| 18 | XX | XX | XX | Do not take triapine on this day |
| 19 | XX | XX | XX | Do not take triapine on this day |
| 20 | XX | XX | XX | Do not take triapine on this day |
| 21 | XX | XX | XX | Do not take triapine on this day |
| 22 | XX | XX | XX | Do not take triapine on this day |
| 23 | XX | XX | XX | Do not take triapine on this day |
| 24 | XX | XX | XX | Do not take triapine on this day |
| 25 | XX | XX | XX | Do not take triapine on this day |
| 26 | XX | XX | XX | Do not take triapine on this day |
| 27 | XX | XX | XX | Do not take triapine on this day |
| 28 | XX | XX | XX | Do not take triapine on this day |

|  |
| --- |
| **Physician’s Office will complete this section:**1. Date patient started protocol treatment \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_2. Date patient was removed from study \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_3. Patient’s planned total daily dose \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_4. Total number of capsules taken this month \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_5. Physician/Nurse/Data Manager’s Signature/Date \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ |

**Patient’s Initials / Date:** \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

# APPENDIX E RANO 2.0 CRITERIA FOR OVERALL RESPONSE STATUS

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| Target Lesiona (current scan) | Target Lesionb (previous scan) | New Measurable Disease | Nontarget or Nonmeasurable Lesion(s) | Clinical Status | Increased Steroid Use | Steroid Dose | Overall Response Status |
| CR | Baseline/SD/PR | No | None/stable | Stable/Improved | Noc | Nonec | Preliminary CR |
| CR | CR | No | None/CR | Stable/Improved | No | None | Confirmed CR |
| CR | Baseline/SD/PR/CR | Yes | None/CR | Stable/Improved | No | None | PDd |
| CR | Baseline/SD/PR/CR | No | None/CR | Worse | No | None | PD |
| CR | Baseline/SD/PR/CR | No | Worsee | Stable/Improved | No | None | PDe |
| CR | Baseline/SD/PR/CR | No | None/CR | Stable/Improved | Yes | Yes | SD or PDf |
| PR | Baseline/SD | No | None/stable | Stable/Improved | Noc | Nonec | Preliminary CR |
| PR | PR | No | None/stable | Stable/Improved | No | None | Confirmed CR |
| PR | Baseline/SD/PR | Yes | None/stable | Stable/Improved | No | None | PDd |
| PR | Baseline/SD/PR | No | None/stable | Worse | No | None | PD |
| PR | Baseline/SD/PR | No | Worsee | Stable/Improved | No | None | PDe |
| PR | Baseline/SD/PR | No | None/stable | Stable/Improved | Yes | Yes | SD or PDf |
| SD | Baseline/SD | No | None/stable | Stable/Improved | Noc | Nonec | SD |
| SD | Baseline/SD | Yes | None/stable | Stable/Improved | No | None | PDd |
| SD | Baseline/SD | No | None/stable | Worse | No | None | PD |
| SD | Baseline/SD | No | Worsee | Stable/Improved | No | None | PDe |
| SD | Baseline/SD | No | None/stable | Stable/Improved | Yes | Yes | SD or PDf |
| PD | Baseline/SD/PR/CR | No | None/stable | Stable/Improved | Noc | Nonec | PD or preliminary PDh |
| PD | Preliminary PDg | No | None/stable | Stable/Improved | No | None | Confirmed PD |
| PD | Baseline/SD/PR/CR | Yes | None/stable | Stable/Improved | No | None | PDd |
| PD | Baseline/SD/PR/CR | No | None/stable | Worse | No | None | PD |
| PD | Baseline/SD/PR/CR | No | Worsee | Stable/Improved | No | None | PDe |
| PD | Baseline/SD/PR/CR | No | None/stable | Stable/Improved | Yes | Yes | PD |

NOTE. For patients who have both enhancing and nonenhancing lesions evaluated, CR and PR for either contrast-enhancing or T2/FLAIR lesions must be accompanied by at least SD in the other lesions. PD in either contrast-enhancing or T2/FLAIR lesions will qualify as progression, regardless of the response in other lesions.

Abbreviations: CR, complete response; FLAIR, fluid-attenuated inversion recovery; MR, minor response; MRI, magnetic resonance imaging; PD, progressive disease; PR, partial response; SD, stable disease.

aContrast-enhancing or non-contrast-enhancing lesion(s) or both depending on the criteria used.

bContrast-enhancing or non-contrast-enhancing lesion(s) or both depending on the criteria used.

cNone or physiologic replacement doses.

dNew sites of measurable disease constitute PD in the case of no measurable disease at baseline or best response. If confirmation scans are required, new sites are added to the sum of bidimensional products or total lesion volume. The new lesion will be considered PD if confirmed by a subsequent scan ≥4 weeks later exhibiting ≥25% increase in sum of perpendicular diameters or ≥40% increase in total volume of enhancing lesions relative to the scan first illustrating new measurable disease.

eProgression of nonmeasurable lesions occurs when lesions that are not measurable become measurable (10 x 10 mm). Nontarget lesions qualify for progression if there is ≥25% increase in area or ≥40% increase in volume. For both nonmeasurable and nontarget lesions, the increase should be added to the sum of the target lesions. The designation of overall progression requires ≥25% increase in sum of products of perpendicular diameters or ≥40% increase in volume of the target lesions together with the nonmeasurable or nontarget lesion(s).

fIncrease in corticosteroid dose alone, in the absence of clinical deterioration related to tumor, will not be used as a determinant of progression. Patients with stable imaging studies whose corticosteroid dose was increased for reasons other than clinical deterioration related to tumor do not qualify for SD or progression. They should be observed closely. If their corticosteroid dose can be reduced back to baseline, they will be considered as having SD; if further clinical deterioration related to tumor becomes apparent, they will be considered to have progression. The date of progression should be the first time point at which corticosteroid increase was necessary.

gOnly relevant when confirmation of progression is required.

hPD if no confirmation of progression required; preliminary PD if confirmation of progression required. If next scan shows SD/PR/CR, then progression is not confirmed and the previous scan showing preliminary PD is noted as pseudo-progression and the patient continued on therapy. The original MRI showing preliminary PD or the second scan, depending on which scan has the smallest sum of the products of the perpendicular diameters or volume, will serve as the baseline for future comparison.

References

1. Wen, P.Y., M. van den Bent, G. Youssef, *et al.* (2023). RANO 2.0: Update to the response assessment in neuro-oncology criteria for high- and low-grade gliomas in adults. *J Clin Oncol.* 41(33):5187-5199.

# APPENDIX F PK Sample Collection Flowsheet (All patients)



# APPENDIX G PK Sample Collection Flowsheet (Microdialysis substudy patients ONLY)



# APPENDIX H Microdialysis Sample Collection Flowsheet (Microdialysis substudy patients ONLY)

**PI: Dr. Yoon: pager – (626) 231-4641; cell – (626) 873-5241**

**Lab director: Dr. Tim Synold; cell – (626) 485-8178; office – (626) 218-1110**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Date** | **Dialysate Sample** | **Sample collection****Frequency** | **\*Actual Time** | **Vial changed by (Initials)** | **Time of triapine administration****(record below)** | **Comments/PK** |
|  | 1 | At start of pump-change q3 hours until triapine administration |  |  |  |  |
|  | 2 |  |  |  |  |  |
|  | 3 |  |  |  |  |  |
|  | 4 |  |  |  |  |  |
|  | 5 |  |  |  |  |  |
|  | 6 |  |  |  |  |  |
|  | 7 |  |  |  |  |  |
|  | 8 | Record time of triapine administration- change vials q1 hour |  |  |  |  |
|  | 9 |  |  |  |  |  |
|  | 10 |  |  |  |  |  |
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|  | 36 |  |  |  |  |  |

**Place Microdialysis Samples on Dry Ice and keep at bedside for pick up by Protocol Nurse**

\* Use **wall clock** to record time (record real time to the nearest minute)