CTEP #9483
Clinical Center #: 13-C-0118

**TITLE:** A Phase I/II Trial of TRC102 (methoxyamine HCl) in Combination with Temozolomide in Patients with Relapsed Solid Tumors and Lymphomas

**Abbreviated Title:** Ph I/II TRC102 Temozolomide


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**NCI Supplied Agent:** TRC102 - NSC 3801

**Commercial Agent:** Temozolomide – NSC 362856

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**Protocol Version Date:** July 6, 2022

## PRÉCIS

**Background**:

- Base excision repair (BER) of DNA repair pathway has been implicated in resistance to both alkylating and antimetabolite chemotherapy.
- TRC102 (methoxyamine HCl) acts through a novel mechanism to inhibit BER and has demonstrated the ability to potentiate the activity of the alkylating agent temozolomide (TMZ), in vitro and in vivo. We hypothesize that TRC102 can be safely co-administered with TMZ and would potentiate DNA damage caused by TMZ, resulting in antitumor responses.
- Based on responses measured during the Phase I portion of the trial, we will further explore the efficacy of this combination in patients with metastatic colon carcinoma, non-small cell lung cancer (NSCLC), and granulosa cell ovarian cancer

**Primary Objective:**

- To establish the safety, tolerability, and maximum tolerated dose (MTD) of oral TRC102 in combination with oral TMZ in patients with refractory solid tumors
- Evaluate the pharmacokinetic (PK) profile of oral TRC102 when administered in combination with TMZ.
- To explore the response rate of this combination in patients with colon cancer, NSCLC, and granulosa cell ovarian cancer

**Secondary Objective:**

- To explore the progression free survival rate of this combination in patients with colon cancer, NSCLC, and granulosa cell ovarian cancer

**Exploratory Objectives:**

- Investigate tumor genomic and transcriptomic alterations potentially associated with sensitivity and/or the development of resistance to TRC102 and temozolomide.
- Determine the effects of the study treatment on the level of histone γH2AX in circulating tumor cells (CTCs) and tumor and correlate the γH2AX response in tumor and CTCs
- Determine the effects of the study treatment on the levels of cleaved caspase 3, epithelial-mesenchymal transition, and APE in tumor and CTCs
- Determine and characterize the effects of study treatment on erythrocytes
- Characterize the clinical presentation of hemolysis observed in earlier study subjects and explore the possible mechanisms

**Eligibility**:

- Phase I: histologically confirmed solid tumors that have progressed on standard therapy known to prolong survival or for which no standard treatment options exist
- Phase II: histologically confirmed adenocarcinoma of the colon post at least two lines of therapy, NSCLC post at least two lines of therapy, or granulosa cell ovarian cancer post at least one line of therapy
- No major surgery, radiation, or chemotherapy within 4 weeks prior to entering the study
- Adequate organ function

- Healthy adult volunteers ≥ 18 years of age will be consented to donate research blood
  ***Please note:*** *As of* ***Amendment R (5/12/18)****, healthy adult volunteers will no longer be recruited to provide blood for this study as we will no longer perform the hemolysis analysis.*

**Study Design**:

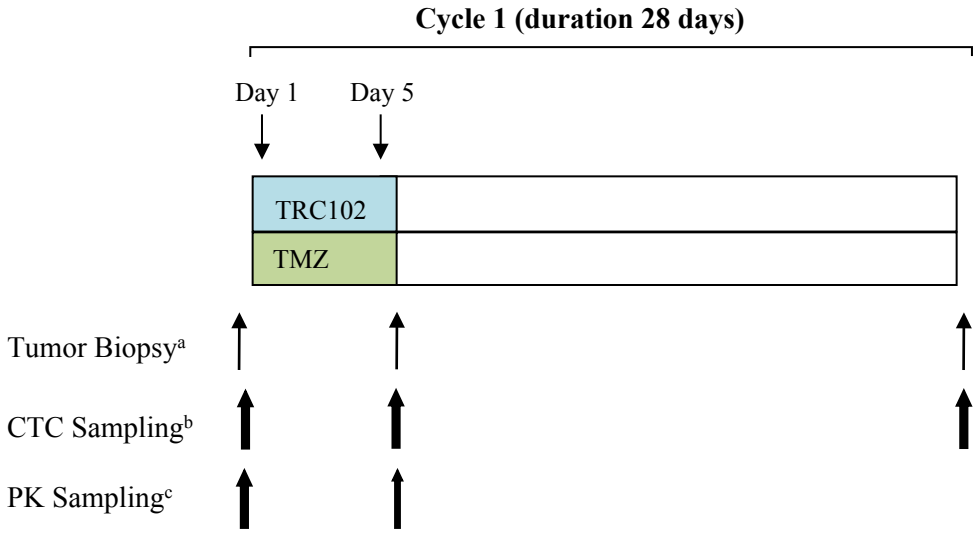
**Phase I**

- This is an open-label Phase I trial; traditional 3+3 design.
- Oral TRC102 and oral TMZ will be administered daily, days 1-5 in 28-day cycles
- Once the MTD is established, up to 15 additional patients will be enrolled at the MTD to further evaluate that dose for PK and PD endpoints for evidence of DNA damage and apoptosis.
- During the escalation phase, tumor biopsies will be optional. During the expansion phase, (once MTD is reached), mandatory paired tumor biopsies will be pursued in the 15 additional patients enrolled to further evaluate PD endpoints.

**Phase II**

- This is a 3-arm Simon 2-stage design trial evaluating independently the response rate of patients with colon, NSCLC, and granulosa cell ovarian cancer.
- Patients with a body surface area (BSA) of ≥1.6 $m^2$ will receive 125 mg of TRC 102 and 150 mg/$m^2$ of TMZ PO qday x 5 every 28 days (DL6). Patients with a BSA of <1.6 $m^2$ will receive 100 mg of TRC 102 and 150 mg/$m^2$ of TMZ PO qday x 5 every 28 days (DL5). Each cycle will be 28 days.
- The accrual ceiling for the Phase II portion is 75 patients.
- Mandatory paired tumor biopsies will be pursued to further evaluate PD endpoints.

## SCHEMA: PHASE I




TRC102 and TMZ will be administered daily on days 1-5

a Biopsies will be optional during the escalation phase and mandatory in the 15 additional patients enrolled at the MTD during the expansion phase. Biopsies will be collected before drug administration on study (baseline); on cycle 1, day 5 (±1 day in case of scheduling difficulties) at 3-4 hrs after drug; and at time of disease progression (optional)

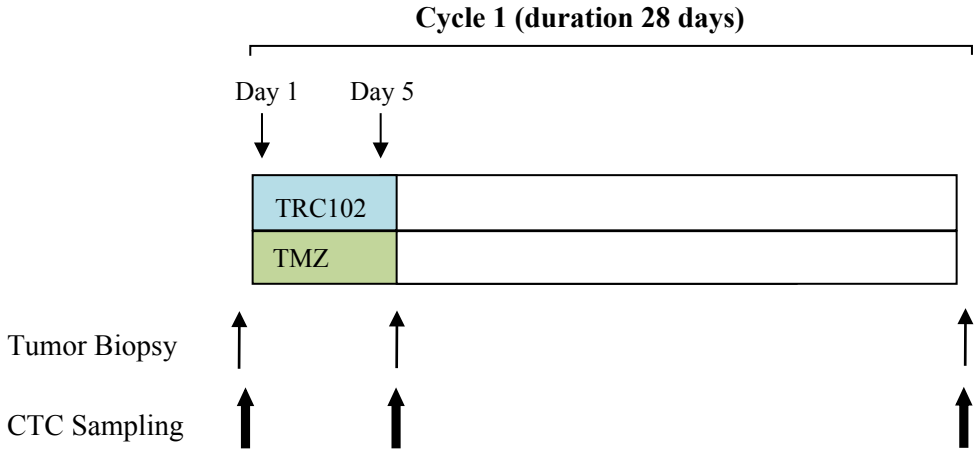
b Blood samples for CTCs (optional) will be collected from all patients at the following time points: cycle 1 prior to treatment, cycle 1 day 1, 8 hrs after drug in both the escalation and expansion phases, cycle 1 day 5 (±1 day in case of scheduling difficulties) *in the expansion phase only*: cycle 2 day 1 and day 1 of all subsequent cycles before drug in both the escalation and expansion phases, and at time of disease progression

c Blood samples for PK analyses (optional) will be collected in Cycle 1 only prior to drug administration and 1, 2, 3, 4, 8, 12, and 24 hours post-dose in both the escalation and expansion phases, and once on day 5 prior to dose in the expansion phase only

| **Phase I Dose Escalation Schedule (28-day cycle)** | | |
|---|---|---|
| **Dose Level** | **TRC102 orally** | **TMZ orally** |
| Level 1 | 25 mg once daily D1-5 | 125 mg/m$^2$ once daily D1-5 |
| Level 2 | 50 mg once daily D1-5 | 125 mg/m$^2$ once daily D1-5 |
| Level 3 | 50 mg once daily D1-5 | 150 mg/m$^2$ once daily D1-5 |
| Level 4 | 75 mg once daily D1-5 | 150 mg/m$^2$ once daily D1-5 |
| Level 5 | 100 mg once daily D1-5 | 150 mg/m$^2$ once daily D1-5 |
| Level 6 | 125 mg once daily D1-5 | 150 mg/m$^2$ once daily D1-5 |
| Level 7 | 150 mg once daily D1-5 | 150 mg/m$^2$ once daily D1-5 |
| Level 8* | 150 mg once daily D1-5 | 200 mg/m$^2$ once daily D1-5 |

*Patients on DL7 can escalate to DL8 from cycle 2 onwards if DL 7 is tolerated in cycle 1

**SCHEMA: PHASE II**




Patients on three separate cohorts (colon cancer, NSCLC, or granulosa cell ovarian cancer) will each receive one of two dose levels:

- patients with a BSA ≥1.6 $m^2$ will receive 125 mg TRC 102 and 150 mg/$m^2$ TMZ po daily on days 1-5 (DL6)
- patients with a BSA <1.6 $m^2$ will receive 100 mg TRC 102 and 150 mg/$m^2$ TMZ po daily on days 1-5 (DL5)

Mandatory biopsies (patients with colon and granulosa cell ovarian cancer only) will be collected before drug administration on study (baseline) and 3-4 hrs after drug administration on cycle 1, day 4 or 5, (post-dose). One optional signal restaging follow-up biopsy may be performed on day 1 (± 2 days) of the cycle following any restaging at which a 10-19% increase in tumor volume is observed (according to RECIST criteria) if the patient has been on study for at least 4 cycles, or at time of disease progression.

Blood samples for CTCs (optional; all patients) will be collected at the following time points: cycle 1 prior to treatment, before treatment on cycle 1 day 4 or 5 (on the day of the post-dose biopsy), day 1 (±1 day) of all subsequent cycles before drug, and at time of disease progression

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# 1. OBJECTIVES

## 1.1 Primary Objectives

- To establish the safety, tolerability, and maximum tolerated dose of oral TRC102 (methoxyamine HCl) in combination with oral temozolomide (TMZ) in patients with refractory solid tumors
- Evaluate the pharmacokinetic (PK) profile of oral TRC102 when administered in combination with TMZ
- To explore the response rate of this combination in patients with colon cancer, NSCLC, and granulosa cell ovarian cancer

## 1.2 Secondary Objective

- To explore the progression free survival rate of this combination in patients with colon cancer, NSCLC, and granulosa cell ovarian cancer

## 1.3 Exploratory Objectives:

- Investigate tumor genomic and transcriptomic alterations potentially associated with sensitivity and/or the development of resistance to TRC102 and temozolomide.
- Determine the effects of the study treatment on the level of histone γH2AX in circulating tumor cells (CTCs) and tumor and correlate the γH2AX response in tumor and CTCs
- Determine the effects of the study treatment on the levels of cleaved caspase 3 and Ki-67 in tumor
- To explore resistance mechanisms to the study drug combination
- Determine and characterize the effects of study treatment on erythrocytes.
- Characterize the clinical presentation of hemolysis observed in earlier study subjects and explore the possible mechanisms

# 2. BACKGROUND

Resistance to chemotherapy is a primary reason that patients fail treatment. Among the various mechanisms by which resistance to chemotherapy can develop, the base excision repair (BER) pathway has been shown to promote resistance to both alkylating and antimetabolite chemotherapy. TRC102 acts through a novel mechanism to inhibit BER and has demonstrated the ability to potentiate the activity of the alkylating agents temozolomide and carmustine, and the antimetabolite agents fludarabine and pemetrexed in murine models of human cancer. Therefore, TRC102 may be able to potentiate the activity of alkylating and antimetabolite chemotherapy [1].

Published studies indicate that TRC102 has the ability to interrupt the process of BER by

binding to apurinic/apyrimidinic (AP) sites produced during the initial step of BER [1, 2]. TRC102-bound AP sites are not substrates for apurinic/apyrimidinic endonuclease (APE), which performs an essential step in BER. TRC102-bound AP sites are, however, substrates for topoisomerase II (topo II), an enzyme that cleaves damaged DNA. *In vitro* studies of cancer cells that contain high levels of topo II indicate that TRC102 effectively potentiates the activity of chemotherapy.

The ability of TRC102 to interrupt the BER pathway was demonstrated *in vitro* [2]. TRC102 was tested for its ability to modify DNA in a way to prevent its cleavage by APE, which hydrolyzes the phosphodiester backbone 5' to the AP site generated by the action of DNA deglycosylase (Figure 1) [1]. An oligonucleotide containing a single uracil base was synthesized and then incubated with uracil deglycosylase (UDG), a DNA deglycosylase, to excise the uracil within the oligonucleotide and produce an AP site. Following the reaction with UDG, the oligonucleotide was incubated with or without TRC102, to produce substrates containing an AP site or an AP site bound to TRC102, respectively. In the absence of TRC102, AP sites were efficiently hydrolyzed by purified APE, demonstrating a cleavage product of lower molecular mass.

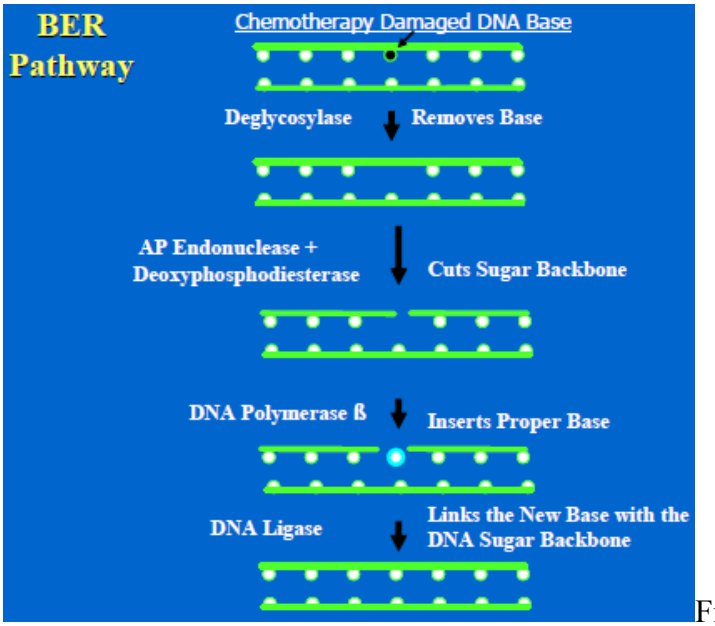


Fig 1. BER pathway.

In contrast, TRC102-bound AP-sites were resistant to the hydrolytic activity of APE, indicating that TRC102-bound AP-sites are not substrates for AP endonuclease and providing a mechanism of action by which TRC102 inhibits the BER pathway (Figure 2). As a correlative study, we will measure AP-site accumulation in DNA at baseline and following TRC102 plus TMZ treatment.

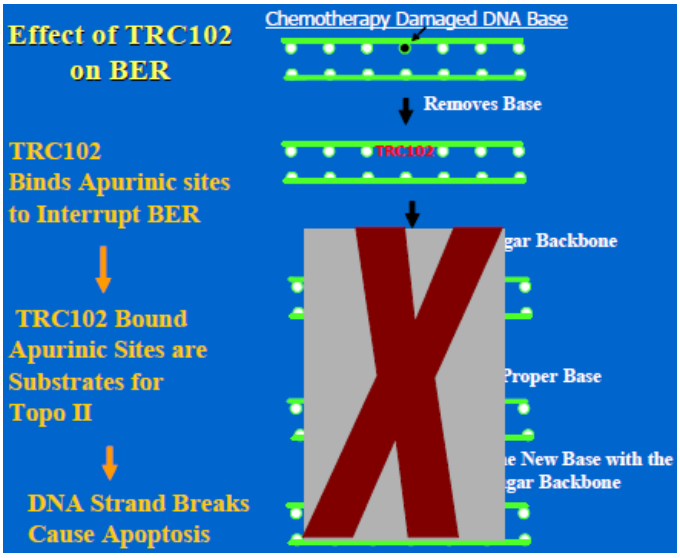



Fig 2. Effect of TRC102 on the BER pathway. TRC102 interrupts BER, because TRC102- bound AP sites are not substrates for APE, a critical BER enzyme. TRC102-bound AP sites, however, are substrates for topo II-mediated DNA cleavage. DNA single and double-strand breaks produced through the action of topo II on TRC102-bound DNA lead to cellular apoptosis.

### 2.1 Background on the Activity of TRC102 in Combination with Alkylating Chemotherapy

The ability of TRC102 to potentiate chemotherapy was initially demonstrated using the alkylating agent temozolomide [3, 4]. Collectively, the available data indicate that treatment of cancer cells with temozolomide produced N7-methylguanine and N3-methyladenine DNA adducts that activated BER to generate AP sites within double-stranded DNA. TRC102 bound covalently to AP sites to form structurally modified AP sites that were then resistant to the repair activity of APE. The persistence of these lesions led to cell death through the generation of DNA strand breaks.

The lethal toxicity induced by the alkylating agent temozolomide combined with TRC102 appears to be mediated through the poisoning effect of topo II [2]. While TRC102-bound AP sites are refractory to the catalytic activity of APE (indicating their ability to block BER), they are cleaved by purified topo II or nuclear extracts from tumor cells expressing high levels of topo II, suggesting that TRC102-bound AP sites stimulate topo II-mediated DNA cleavage [2]. Furthermore, cells treated with temozolomide and TRC102 demonstrate increased expression of topo II and increased formation of phosphorylated histone H2AX (γH2AX), a marker of DNA double strand breaks, that co-localizes with up-regulated topo II. These data support the hypothesis that DNA double-strand breaks marked by γH2AX foci are associated with topo II in cells.

The cleavage of TRC102-bound AP sites by topo II also explains the ability of TRC102 to potentiate the effects of temozolomide in cancer cells (that have high levels of topo II) compared to normal cells (which have low levels of topo II). For example, *in vitro* addition of TRC102 effectively sensitized tumor cells, but not bone marrow cells, to the effects of temozolomide (Figure 3).

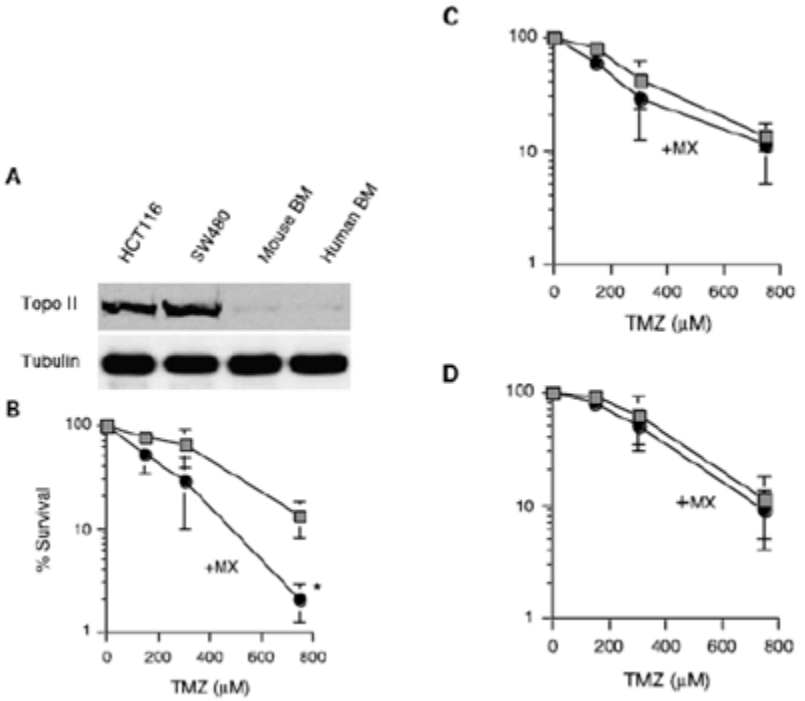



Fig 3. Levels of topo II are increased in human colon HCT116 and SW480 cancer cell lines, but not in bone marrow cells by Western blot (Panel A). TRC102 (MX) potentiates the *in vitro* activity of temozolomide in inhibiting survival of SW480 human colon cancer cells (Panel B) but has minimal effect on temozolomide activity towards human or murine bone marrow cells (Panels C and D, respectively) [2].

In murine xenograft studies, TRC102 efficiently potentiated the antitumor effect of temozolomide and carmustine in several colon cancer cell lines regardless of cell line genetic status, including O6-methylguanine DNA-methyltransferase (MGMT), mismatch repair (MMR), or p53 status [3]. For example, the combination of temozolomide and TRC102 caused regression of SW480 tumor xenografts that were resistant to treatment with temozolomide alone. Importantly, treatment with temozolomide plus TRC102 did not result in higher levels of myelosuppression or weight loss compared to treatment with temozolomide alone [3].

## 2.2 TRC102

### Clinical Experience

### IV TRC102 with Oral TMZ

Initial pharmacokinetic (PK) analyses of patients receiving TRC102 as a 5-day continuous infusion revealed a distinct PK profile, 10-fold greater than previously estimated in dogs, where half-life of TRC102 was estimated to be 4.5 hours. As a result, TRC102 administration adjusted from a five-day continuous infusion to a one hour intravenous infusion. Twenty-five patients have enrolled, in two cohorts. In cohort A (patients with no-CNS disease), patients have enrolled in dose-levels 1, 2 and 3 (TMZ 150 mg/m$^2$/day, days 1-5 and TRC102, 15 mg/m$^2$, 30 mg/m$^2$ and 60 mg/m$^2$ respectively). In cohort B (patients with CNS involvement), patients

have enrolled in dose-levels 1 and 2. Enrollment started at a DL lower (in DL1, TMZ 100 mg/m$^2$/day, days 1-5 and TRC102, 15 mg/m$^2$; for DL2 TMZ increased to 150 mg/m$^2$/day, days 1-5 and TRC102 remained 15 mg/m$^2$). The average half-life of TRC102 administered as an one-hour continuous infusion was 55.04 hours (range: 12.2 - 100.3 hours, n = 20), statistically not different from the half-life of TRC102 administered as a 5-day continuous infusion which was 45.1 hours (range: 32.1 - 68.8 hours, n = 6). Pharmacodynamic (PD) results from 22 patients showed that administration of the combination of TMZ and TRC102 resulted in 10-40% reduction in detectable AP sites. Comet assay results from 20 patients revealed that the combination of TMZ and TRC102 induced a 2 to 3-fold higher levels of DNA strand breaks compared to TMZ alone. PD demonstration of TRC102's biologic activity on patients' mononuclear cells has been demonstrated even at the lowest DL. The combination of TRC102 and TMZ has been well-tolerated. For the non-CNS involvement cohort, one DLT was observed at DL1; grade 3 psychosis in a patient with progressive disease on increasing doses of opioids. A grade 3 allergic reaction classified as an idiosyncratic event resulted in further expansion of the cohort to 10 evaluable patients with no additional DLTs observed. No DLTs have been observed at DL2. For the CNS involved cohort, no DLTs have been observed to date. Three patients had stable disease (lung, ovarian, head and neck primary) [5].

**Oral TRC102 with IV Pemetrexed**

The safety, PK, and PD of oral TRC102 combined with IV pemetrexed was evaluated. TRC102 alone was administered on days 1- 4 of a 2-week cycle followed by TRC102 (D1-4) and 500 mg/m$^2$ pemetrexed (D1) every 3 weeks thereafter. Twenty eight patients were enrolled and treated with a total of 93 cycles of TRC102 at 15 mg/m$^2$ (n=4), 30 mg/m$^2$ (n=7), 60 mg/m$^2$ (n=11) and 100 mg/m$^2$ (n=6). The MTD was exceeded at 100 mg/m$^2$ due to grade 3 anemia in 50% of patients. No other DLTs were reported. Other adverse events possibly related to TRC102 included grade 3/4 neutropenia without fever, grade 3 thrombocytopenia, grade 1/2 fatigue, and grade 1/2 asthenia. TRC102 plasma concentrations required for in vivo activity were achieved at all dose levels studied (Cmax > 50 ng/mL, t1/2 = 28 hr). PD studies confirmed that TRC102 binds pemetrexed-induced AP sites. One patient at 30 mg/m$^2$ TRC102 with oropharyngeal adenoid cystic cancer metastatic to the lung had RECIST-defined PR that lasted until cycle 14. Stable disease for ≥ 3 cycles was seen in 3 patients at 30 mg/m$^2$, 2 patients at 60 mg/m$^2$, and 4 patients at 100 mg/m$^2$, including one patient with squamous cell lung cancer who had SD for 9 cycles. So this study showed that a daily oral TRC102 is well-tolerated at doses expected to inhibit BER and potentiate the activity of chemotherapy. The recommended phase II dose is 60 mg/m$^2$ p.o. for 4 days in combination with standard dose pemetrexed [5, 6].

### 2.3 Temozolomide (TMZ)

TMZ is an alkylating drug approved by the FDA (http://www.accessdata.fda.gov) for the treatment of adult patients with newly diagnosed glioblastoma multiforme (GBM) concomitantly with radiotherapy and then as maintenance treatment and refractory anaplastic astrocytoma patients who have experienced disease progression on a drug regimen containing nitrosourea and procarbazine. TMZ is not directly active but undergoes rapid nonenzymatic conversion at physiologic pH to the reactive compound 5-(3-methyltriazen-1-yl)-imidazole-4-carboxamide (MTIC). The cytotoxicity of MTIC is thought to be primarily due to alkylation of DNA. Alkylation (methylation) occurs mainly at the O6 and N7 positions of guanine. TMZ is

rapidly and completely absorbed after oral administration with a peak plasma concentration (Cmax) achieved in a median Tmax of 1 hour. Food reduces the rate and extent of temozolomide absorption. Cytochrome P450 enzymes play only a minor role in the metabolism of temozolomide and MTIC.

**Safety of TMZ**
The most common adverse reactions (≥10% incidence) are alopecia, fatigue, nausea, vomiting, headache, constipation, anorexia, convulsions, rash, hemiparesis, diarrhea, asthenia, fever, dizziness, coordination abnormal, viral infection, amnesia, and insomnia. The most common Grade 3 to 4 hematologic laboratory abnormalities (≥10% incidence) that have developed during treatment with temozolomide are lymphopenia, thrombocytopenia, neutropenia, and leucopenia which requires monitoring absolute neutrophil count (ANC) and platelet count prior to dosing and throughout treatment. Cases of myelodysplastic syndrome and secondary malignancies, including myeloid leukemia, have been observed. All patients, particularly those receiving steroids, should be observed closely for the development of lymphopenia and *Pneumocystis jiroveci* pneumonia. Cases of hepatic injury, including fatal hepatic failure, have also been observed in patients enrolled on temozolomide studies; it was noted that liver toxicity may occur several weeks or more after initiation of treatment or after temozolomide discontinuation.

**Current Protocol Status**
As of October 2017, we have completed accrual to the Phase 1 portion of this study (52 patients); DL7 was established as the MTD after two DLTs occurred on DL8 (Grade 3 hemolysis and abdominal pain). However, because 6 patients had grade 3 anemia requiring transfusion in the DL7 expansion cohort, subsequent patients were treated at DL6 (125 mg TRC 102, 150 mg/m$^2$ TMZ) and none of the remaining Phase I patients on DL6 required transfusions.

In the Phase I portion of the trial there were four confirmed PRs (one colon, one NSCLC, two granulosa cell ovarian cancer) and one unconfirmed PR (colon); 11 patients have had stable disease. All patients had prior therapy. The one unconfirmed PR had stroke that was not considered to be drug related prior to confirmation; this patient came off study at the time of stroke, but returned 2 years later and received an additional three cycles of TRC 102 plus temozolomide on Special Exception protocol E16-5001 (16-C-9950) at the Clinical Center at a dose of 125 mg TRC 102 and 150 mg/m$^2$ TMZ before coming off treatment per patient decision.

We began accruing to the Phase II portion of the trial in February of 2016 at DL6 and have accrued 26 of patients as of October 20, 2017. Due to a recent grade 3 anemia requiring RBC transfusion in a patient with very low BSA (1.43 m$^2$) receiving DL6, as of **Amendment P** (10/20/2017) we will administer a maximum of 100 mg TRC102 daily (DL5) for patient whose BSA is lower than 1.6. Patients with BSA equal or higher than 1.6 will receive a maximum of 125mg TRC102 daily (DL6).

Of note, analysis of the pharmacokinetic data collected to date indicate that all dose levels of TRC102 reached Cmax >50 ng/mL required for in vivo activity in preclinical models, and that

combining these two agents does not alter the pharmacokinetics of either. Due to prior suggestions of anemia, patients on the expansion cohort will undergo a hemolysis workup, which is optional and as clinically indicated.

### 2.4 Rationale

TRC102 has been shown to potentiate the activity of temozolomide by preventing BER and allowing cleavage of TRC102 bound DNA, which will cause DNA strand breaks in cancer cells. We hypothesize that oral TRC102 can be safely co-administered with TMZ and would potentiate DNA damage caused by TMZ resulting in antitumor responses. Based on drug mechanisms of action we anticipate that patient responses will be associated with an MGMT-low/negative + DNA mismatch repair-competent phenotype, and that eventual resistance to study drugs will be associated with the loss of this DNA repair phenotype.

Granulosa cell tumor of the ovary is a stromal cell neoplasm that is able to secrete sex steroids such as estradiol, inhibin, follicle regulatory protein, and mullerian inhibitory substance, all of which have been investigated as prognostic markers [7-11]. Granulosa cell tumors make up approximately 2-5% of ovarian cancers [12]. They are very rare and have a long natural history as well as tendency to recur years after their diagnosis [13]. Advanced metastatic disease is present in about 10% of cases. Average age of presentation is perimenopause or postmenopausal women around 50 years [14, 15]. Initial treatment is similar to that for epithelial ovarian cancer with surgery as the backbone in early stage disease. Radiation has also been used but there is not sufficient data for its use in the adjuvant setting [16]. Chemotherapy has been shown to be effective in series of case series both with combinations including BEP [17], PVB [18] as well as single agents including paclitaxel [19] and tamoxifen [20].

Lung cancer has the highest mortality rate among cancers worldwide. Lung cancer remains the leading cause of cancer-related mortality in the United States although its incidence is decreasing. In 2014, 224,210 new cases are projected and 146,409 persons are calculated to die from the disease in the United States [21]. Chemotherapy combined with surgery and radiotherapy are the mainstays for many patients treated with lung cancer although many feel that these strategies alone have reached a therapeutic plateau. Changes in major cell-signaling and regulatory pathways are now thought to play a major role in tumorigenesis. Small molecule targets toward specific targets (e.g., ALK, EGFR, ROSS1, ERCC1) as well as immunotherapy are being used and further explored alone and in combinations with traditional therapies[22]. Long term survival remains poor for all patients but especially for those patients who are diagnosed with metastatic disease and new avenues need to be found to continue to improve outcomes. There is no expectation that there will be a different expectation of response in adenocarcinoma vs. squamous cell lung cancer; we are planning to accrue both populations.

Colorectal cancer (CRC) is one of the most prevalent cancers and a leading cause of cancer mortality worldwide [21]. To better understand the biologic markers of the disease, CRC has undergone extensive molecular characterization, which revealed important oncogenes (e.g., KRAS, BRAF, PIK3CA), tumor suppressor genes (e.g., APC, TP53, PTEN) and signaling pathways that are critical for the development, survival, and progression these cancer cells [23]. These genes are involved in major signaling pathways that have been linked to cancer,

including the WNT/β-catenin, mitogen-activated protein kinase (MAPK), transforming growth factor beta (TGF-β) and phosphoinositide 3-kinase (PI3K) pathways [24]. A number of targeted agents against specific genomic targets have been developed. Patients whose tumors are characterized based on these molecular markers still show remarkable variability in terms of prognosis and response to therapy [25]. Many studies have addressed sub-classification of CRC, focusing on epigenetic factors and gene expression profiles to help guide clinical treatment and practice [26]. Colorectal cancer patients with known MSI-high disease who have not been previously treated with immunotherapy will be excluded from the Phase II portion of this study based on reports that they may benefit from PD-1 blockade therapy [27].

Partial responses in granulosa cell ovarian cancer (2 patients), NSCLC (1 patient with squamous cell), and colorectal cancer (one almost complete response and one unconfirmed) seen in the Phase 1 trial warrants further investigations in terms of response and identifying predictive biomarkers. There are no readily available molecular or cytological links that would predict the response in these distinct tumor types.

### 2.5 Correlative Studies Background

Tumor tissue biopsies and circulating tumor cells will be collected to measure levels of phosphorylated histone H2AX (γH2AX), one of the earliest markers of DNA double-strand breaks [28-30]. H2AX is phosphorylated at its C-terminus (serine 139 in humans) within minutes following DNA double-strand breaks marking the chromatin domain around the broken chromosomal DNA ends, thus allowing the recruitment of repair factors [28, 30-33]. Levels of γH2AX within a cell are directly correlated to the number of DNA double-strand breaks. Hence γH2AX can be used as a dosimeter and biomarker for DNA double-strand break damage. The Pharmacodynamic Assay Development and Implementation Section (PADIS) at the Frederick National Laboratory for Cancer Research (FNLCR) has validated an immunofluorescent assay for γH2AX [34]. Biopsies will be optional in the escalation phase and mandatory for the expansion phase.

In Phase I, a biopsy will be obtained at baseline (prior to drug administration), 3-4 hours post dose administration on day 5, and optionally at the time of disease progression. Biopsies will only be obtained on patients with easily accessible, biopsiable disease in escalation phase. All patients in the expansion phase should have safely biopsiable disease and be willing to undergo tumor biopsy.

Biopsies are optional in the escalation phase, but with Amendment K, we revised the protocol to consent current patients who are responding (i.e., stable disease or partial response) to consider providing archival tissue, such as a diagnostic tissue block or previously collected biopsy tissue sent for CLIA genetic testing (biopsy core or 6 unstained and one H&E slide). In the absence of this tissue, we would ask these patients to consider consenting to having a research biopsy on study, if the patient has biopsiable disease, with a second optional biopsy at time of disease progression. While having pre-study biopsy tissue is optional for assessing drug response, provision of "on-study" biopsies would address whether drug resistance and eventual loss of response are associated with loss of the MGMT-low/negative plus DNA mismatch repair-

competent phenotype. No more than three on-study biopsies will be requested, and no biopsy will be requested unless there is a reasonable possibility that a paired sample (biopsy or archival tissue) is or will be available.

In Phase II, a biopsy will be obtained at baseline (prior to drug administration) and 3-4 hours post dose administration on day 4 or 5. An optional biopsy will also be collected at the time of disease progression or at the beginning of the cycle following any restaging at which a 10-19% increase in tumor volume is observed (according to RECIST criteria) if the patient has been on study for at least 4 cycles to study drug resistance mechanisms. Biopsies will only be obtained from patients with colon cancer or granulosa cell ovarian cancer. All patients in the expansion phase should have safely biopsiable disease and be willing to undergo tumor biopsy.

Blood sampling for CTCs will be performed for all patients as described in Section 9.2.2.1. We will also evaluate whether we can measure changes in the number and phenotype (epithelial-mesenchymal transition) in CTCs and tumor tissue in patients over time to explore any correlation with response to treatment or disease progression. This analysis will be performed with the ApoStream instrument, which uses antibody-independent CTC isolation technology that can isolate viable CTCs from epithelial and non-epithelial cancers.

We additionally plan to measure cleaved caspase 3 (cCasp3) as a marker of apoptosis. Cleaved caspase 3 is a key protease activated during early stages of apoptosis via cleavage of its pro-enzyme form into a heterodimer of 17- and 12-kDa subunits. Immunofluorescence assays for this marker has been developed and validated by Dr. Kinders' laboratory at FNLCR.

FNLCR has developed an immunoassay for tumor MGMT protein expression to investigate the relative importance of MGMT status for this combination treatment. Additionally, the laboratory of Dr. Stanton Gerson (Case Western Reserve University) has agreed to assay any remaining tumor tissue via IHC for the enzyme DNA-3-methyladenine glycosylase, also known as 3-alkyladenine DNA glycosylase (AAG) or N-methylpurine DNA glycosylase (MPG), the only glycosylase identified to date that excises alkylation-damaged purine bases in humans, such as those produced by temozolomide [35, 36]. No patient data will accompany any samples sent to Dr. Gerson's lab.

While treatment with the combination of TRC102 and temozolomide has been observed to lead to clinical responses in patients on the phase I portion of this trial, patients who respond are likely to eventually progress due to acquired drug resistance, making further understanding of the determinants and mechanisms of acquired resistance a priority. To address this, after **Amendment R** (5/12/2018), the optional tumor biopsies collected at progression or restaging follow-up (i.e., after a restaging at which a 10-19% increase in tumor size is observed) will be sequenced with the CLIA-certified OCAv3 assay by the CLIA-certified Molecular Characterization (MoCha) Laboratory at the Frederick National Laboratory for Cancer Research. Patients undergoing these optional biopsies will receive the Oncomine report from OCAv3 to share with their health care providers in order to help guide future treatment decisions. Genetic information from the tumor at the time of progression will be compared with baseline tumor genetic information to investigate the development of resistance to the combination of TRC102 and temozolomide by mechanisms such as inactivation of mismatch repair genes or the development of increased mutational load and neoantigen production [37].

MoCha will also perform exploratory whole-exome sequencing (WES) and whole transcriptome RNASeq gene expression assays on baseline tumor and germline tissues from patients to attempt to identify genomic alterations and gene expression patterns potentially associated with sensitivity to treatment, the results of which will not be shared with the patients.

Peripheral blood sampling for assessment of anemia and hemolysis will be performed for all patients at baseline and as described in Appendix F. In preclinical animal models, TRC102 caused dose-dependent extravascular hemolysis, as demonstrated by development of anemia, elevation in serum bilirubin, reticulocytosis, and bone marrow hypercellularity reflective of regenerative response, and splenomegaly [Investigator Brochure]. The combination of TRC102 and pemetrexed or TMZ did not affect hematologic changes, and there was no evidence of cumulative effects on hematological toxicity. In a phase I study of TRC102 in combination with pemetrexed for refractory cancer treatment, anemia secondary to extravascular hemolysis was the only dose-limiting toxicity observed [38]. The anemia was reversible and manageable with standard supportive care. The mechanisms of extravascular hemolysis associated with TRC102 are not well understood. In the present trial, hemolytic anemia was the dose-limiting toxicity, and MTD was exceeded at dose-level 8 (TRC102 150 mg, TMZ 200 mg/m$^2$). Given negativity of direct Coombs tests in these patients who experienced hemolytic anemia, hemolytic anemia is considered to be non-immune mediated. An in vitro study of hydroxylamines in human erythrocytes suggests impairment of RBC enzymes as the potential mechanism of hemolysis [39]. We hypothesize that TRC102 induces extravascular hemolysis by affecting erythrocyte enzymes and/or erythrocyte membrane and osmotic tolerance. By identifying the underlying mechanisms and susceptible subjects with underlying RBC enzymopathies or RBC membrane disease, we aim to improve the clinical safety of TRC102. To perform this analysis, healthy adult volunteers will be accrued to provide normal research blood samples. *Please note: As of **Amendment R (5/12/18)**, healthy adult volunteers will no longer be recruited to provide blood for this study as we will no longer perform this analysis of the mechanisms of extravascular hemolysis.*

### 2.6 Genetic Sequencing Research Ethics

This trial will collect identifiable genetic data from patients enrolled after **Amendment R** (5/12/2018) for exploratory studies to examine genomic alterations and gene expression patterns potentially associated with sensitivity and/or acquired resistance to treatment. As patient clinical response data (both for this study and, potentially, after a patient is off-study) will be required for comparison to sequencing results, de-identifying the samples is not feasible. Designing the study therefore poses challenging questions about informed consent, the privacy of the patient and the patient's family, the researchers' obligation to disclose genetic information to the patient, and the use and storage of research data [40-42]. In the vast majority of cases, we do not know the medical significance of genetic variants [43, 44]. These challenges will continue to be evaluated to maintain the rigor and integrity of the study and the wellbeing of our patients. A Certificate of Confidentiality has been obtained from the NIH to help protect the privacy of all study participants.

The informed consent document for this protocol after **Amendment R** (5/12/2018) contains language informing patients about the performance of genetic studies, and patients will have the

option to choose whether they wish to take part in these studies.

Targeted sequencing (CLIA-certified) and whole-exome sequencing (performed for research purposes but non-CLIA) of tumor and blood can detect non-ambiguous germline variants, which may raise health and privacy implications for the patient and his or her family. WES will not be validated for clinical use, and no clinical decisions can be made based on its results. Furthermore, no WES genetic data will be shared with the patient.

This study does not meet the criteria specified by the NIH Genomic Data Sharing (GDS) Policy (see Section 12.4), and therefore, a GDS plan has not been included in this protocol.

## 3. PATIENT SELECTION

### 3.1 Eligibility Criteria (Patients)

3.1.1 Phase I: histologically confirmed solid tumors that have progressed on standard therapy known to prolong survival or for which no standard treatment options exist.

3.1.2 Phase II: histologically confirmed colorectal adenocarcinoma post at least two lines of therapy, NSCLC post at least two lines of therapy, or granulosa cell ovarian cancer post at least one line of therapy. Patients must have measurable disease.

3.1.3 Age ≥18 years. Because no dosing or adverse event data are currently available on the use of TRC102 in combination with TMZ in patients < 18 years of age, children are excluded from this study.

3.1.4 Patients enrolling in the expansion cohorts must have disease amenable to biopsy and be willing to undergo pre-and post-treatment biopsies.

3.1.5 ECOG performance status ≤ 2 (Phase I), ≤ 1(Phase II).

3.1.6 Life expectancy of greater than 3 months

3.1.7 Patients must have normal organ and marrow function as defined below:

| | |
|---|---|
| – Absolute neutrophil count | ≥1,500/mcL |
| – Hemoglobin | ≥10 g/dL without transfusion within 4 days prior to enrollment |
| – Platelets | ≥100,000/mcL |
| – Total bilirubin | ≤1.5 X institutional ULN |
| – AST(SGOT)/ALT(SGPT) | ≤3 X institutional upper limit of normal; 5.0 x ULN in cases of liver metastases |
| – creatinine | ≤1.5 X institutional ULN<br>OR |
| – creatinine clearance | ≥60 mL/min/1.73 $m^2$ for patients with creatinine levels >1.5 mg/dL |

3.1.8 The effects of study drug on the developing human fetus are unknown. For this reason, 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 and for the duration of study participation and for at least 3 months after dosing with study drugs ceases. 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 3 months after completion of study drug administration.

3.1.9 Patients must have completed any chemotherapy, radiation therapy, or biologic therapy ≥ 4 weeks (or 5 half-lives, whichever is shorter) prior to entering the study (6 weeks for nitrosoureas or mitomycin C). Patients must be ≥ 2 weeks since any prior administration of a study drug in a Phase 0 or equivalent study and ≥ 1 week from palliative radiation therapy. Patients must have recovered to eligibility levels from prior toxicity or adverse events. Treatment with bisphosphonates is permitted.

3.1.10 Patients must be able to swallow whole tablets or capsules; nasogastric or G-tube administration is not allowed.

3.1.11 Ability to understand and the willingness to sign a written informed consent document and to undergo tumor biopsies in the expansion phase.

### 3.2 Exclusion Criteria (Patients)

3.2.1 Patients who are actively receiving any other investigational agents.

3.2.2 Patients with active brain metastases or carcinomatous meningitis are excluded from this clinical trial. Patients with treated brain metastases, whose brain metastatic disease has remained stable for ≥ 4 weeks without requiring steroid and anti-seizure medications are eligible to participate.

3.2.3 Phase II only: No other prior malignancies are allowed except for the following:

- Adequately managed stage 0 (carcinoma in situ), I, or II basal cell or squamous cell carcinoma from which the patient is currently in complete remission.
- Any other cancer from which the patient has been disease-free for three years.
- Adequately managed stage I or II well differentiated thyroid or prostate cancer is also eligible, wherein the patient is not required to be in complete remission.

3.2.4 Phase II only: patients with colorectal cancer with known MSI-high disease who have not previously been treated with immunotherapy or who have refused treatment with immunotherapy.

3.2.5 History of allergic reactions attributed to compounds of similar chemical or biologic

composition to TRC102 or TMZ.

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

3.2.7 Pregnant women are excluded from this study because the effects of the study drugs on the developing fetus are unknown. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with the study drugs, breastfeeding should be discontinued prior to the first dose of study drug and women should refrain from nursing throughout the treatment period and for 3 months following the last dose of study drug.

3.2.8 HIV-positive patients on combination antiretroviral therapy are ineligible because of possible PK interactions with TRC102.

### 3.3 Eligibility Criteria (Healthy volunteer blood donors)

*Please note: As of **Amendment R** (5/12/18), healthy adult volunteers will no longer be recruited to provide blood for this study as we will no longer perform the hemolysis analysis.*

3.3.1 Age >18 years; hemoglobin ≥12 g/dL; no history of bleeding problems; not taking aspirin or any medication that may affect erythrocyte biochemistry

3.3.2 Willingness to sign the healthy volunteer informed consent form.

### 3.4 Screening Evaluation

3.4.1 Histologic confirmation: All patients are required to have histologically confirmed solid tumor(s) per Sections 3.1.1 or 3.1.2. Pathology confirmation should preferably be done by the Laboratory of Pathology, NIH. However, pathology reports from outside institutions confirming the diagnosis will also be acceptable for eligibility purposes.

3.4.2 History and physical examination: Complete history and physical examination (including height, weight, vital signs, performance score, EKG) will be conducted within 8 days prior to enrollment.

3.4.3 Imaging Studies (Baseline): Every participant should have an evaluation of known sites of disease as part of the baseline evaluation. All patients will be required to undergo a CT scan of the chest/abdomen/pelvis to evaluate sites of disease within 28 days prior to enrollment. In an occasional patient, MRI may be performed for a disease site instead of a CT scan if MRI is felt to provide more accurate measure of disease. MRI or CT scan with contrast of the brain, MRI liver, MRI for other disease sites, or bone scan may be done as clinically indicated.

3.4.4 Laboratory Evaluation: Baseline laboratory data are to be obtained within 8 days prior to enrollment:

- Hematological Profile: CBC with differential.
- Biochemical Profile: albumin, alkaline phosphatase, total bilirubin, BUN, calcium, creatinine, phosphorus, total protein, SGOT[AST], SGPT[ALT], magnesium, potassium, LDH, and sodium.
- Coagulation Profile: PT, PTT, INR for patients undergoing tumor biopsy.
- Urine and/or serum pregnancy test for female participants of childbearing potential.
- Erythrocyte analyses per Appendix F.

## 4. REGISTRATION PROCEDURES

### 4.1 Patient Registration Process

Registration and status updates (e.g., when a participant is taken off protocol therapy and when a participant is taken off-study) will take place per CCR SOP ADCR-2, CCR Participant Registration & Status Updates found here: https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=73203825

**Cohorts:**

Cohort 1: healthy volunteers
Cohort 2: phase I solid tumor
Cohort 3: phase II CRC
Cohort 4: phase II NSCLC
Cohort 5: phase II granulosa cell

**Arms:**
Arm 1: TRC102 + Temozolomide
Arm 2: no treatment

Cohorts 2, 3, 4, and 5 are assigned to arm 1. Cohort 1 is assigned to arm 2.

### 4.2 Investigator and Research Associate Registration with CTEP

Food and Drug Administration (FDA) regulations require IND sponsors to select qualified investigators. NCI policy requires all persons participating in any NCI-sponsored clinical trial to register and renew their registration annually. To register, all individuals must obtain a CTEP Identity and Access Management (IAM) account (https://ctepcore.nci.nih.gov/iam). In addition, persons with a registration type of Investigator (IVR), Non-Physician Investigator (NPIVR), or Associate Plus (AP) (i.e., clinical site staff requiring write access to OPEN or RAVE or acting as a primary site contact) must complete their annual registration using CTEP's web-based Registration and Credential Repository (RCR) (https://ctepcore.nci.nih.gov/rcr). Documentation requirements per registration type are outlined in the table below.

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

An active CTEP-IAM user account and appropriate RCR registration is required to access all CTEP and CTSU (Cancer Trials Support Unit) websites and applications. In addition, IVRs and NPIVRs must list all clinical practice sites and IRBs covering their practice sites on the FDA Form 1572 in RCR to allow the following:

- Added to a site roster
- Assigned the treating, credit, consenting, or drug shipment (IVR only) tasks in OPEN
- Act as the site-protocol PI on the IRB approval
- Assigned the Clinical Investigator (CI) role on the Delegation of Tasks Log (DTL).

Additional information can be found on the CTEP website at https://ctep.cancer.gov/investigatorResources/default.htm. For questions, please contact the RCR ***Help Desk*** by email at RCRHelpDesk@nih.gov

## 5. TREATMENT PLAN

Phase I: This is an open-label Phase I trial evaluating the combination of oral TRC102 with oral TMZ, in 28-day cycles. The starting dose of TRC102 is based on the safety information from the trial of IV TRC102 combined with TMZ. The study will follow a 3+3 design: dose will be escalated in cohorts of 3 patients with the individual dose of TRC102 and TMZ increased in successive dose levels as outlined in the dose escalation table below. Once the MTD is established, up to 15 additional patients will be enrolled at the MTD (expansion phase) to further define the dose and evaluate PK and PD at this dose level.

**Phase II:** Patients with a body surface area (BSA) of $\geq$1.6 $m^2$ will be started on 125 mg TRC 102 and 150 mg/$m^2$ Temozolomide orally on days 1-5 of each 28-day cycle (DL6). Patients with a BSA of <1.6 $m^2$ will be started on 100 mg TRC 102 and 150 mg/$m^2$ Temozolomide orally on days 1-5 of each 28-day cycle (DL5). There will be no dose escalation for patients on the Phase II portion of the study; appropriate dose modifications are described in Section 6. Up to 75 patients will be accrued to this part of the study.

History and physical examination can be done up to 8 days before start of a new cycle. Patients will be examined at baseline for the first cycle then at the start of every cycle for the remainder of the study (up to 3 days before start of a new cycle). The start of the next cycle may be delayed for up to 1 week to accommodate scheduling conflicts.

Labs (CBC with differential; serum chemistries) will be performed (+/- 2 days) as follows: every week up to week 3 for cycle 1. Starting with cycle 2, labs (CBC with differential; serum chemistries) will only be performed before the start of each cycle (laboratory evaluation may be performed up to 7 days prior to scheduled date to accommodate scheduling conflicts and patient convenience). If clinically indicated, labs may be obtained more frequently.

Blood for erythrocyte analysis (optional) will be collected on cycle 1 days 7, 14, and 21 (+/- 2 days) as described in Appendix F.

CT scans will be performed at baseline, and repeat scans will be performed every 2 cycles (every 3 cycles for patients on study more than one year).

| **Phase I Dose Escalation Schedule (28-day cycle)** | | |
|---|---|---|
| **Dose Level** | **TRC 102 orally** | **TMZ orally** |
| Level -2 | 25 mg once daily D1-5 | 75 mg/m$^2$ once daily D1-5 |
| Level -1 | 25 mg once daily D1-5 | 100 mg/m$^2$ once daily D1-5 |
| Level 1 | 25 mg once daily D1-5 | 125 mg/m$^2$ once daily D1-5 |
| Level 2 | 50 mg once daily D1-5 | 125 mg/m$^2$ once daily D1-5 |
| Level 3 | 50 mg once daily D1-5 | 150 mg/m$^2$ once daily D1-5 |
| Level 4 | 75 mg once daily D1-5 | 150 mg/m$^2$ once daily D1-5 |
| Level 5 | 100 mg once daily D1-5 | 150 mg/m$^2$ once daily D1-5 |
| Level 6 | 125 mg once daily D1-5 | 150 mg/m$^2$ once daily D1-5 |
| Level 7 | 150 mg once daily D1-5 | 150 mg/m$^2$ once daily D1-5 |
| Level 8* | 150 mg once daily D1-5 | 200 mg/m$^2$ once daily D1-5 |

*Patients on DL7 can escalate to DL8 from cycle 2 onwards if DL 7 is tolerated in cycle 1

Tumor biopsies will be optional during the escalation phase. For the escalation phase, 3 patients should have completed at least one cycle of therapy prior to considering dose escalation in the next cohort of patients. Dose escalation will proceed according to the dose escalation table. DLT is defined in Section 5.2. Determination of DLT will be based on toxicities observed in the first cycle of therapy. Patients are considered evaluable for toxicity for the purpose of cohort dose escalation decisions if they either 1) experienced DLT or 2) have received at least 90% of the planned 28-day doses of treatment in one cycle of therapy and have been followed for one full cycle without DLT. All toxicities will be reported for all patients who receive any amount of study drug on this study. Evaluation of toxicity will begin with study drug administration on cycle 1 day 1.

Intra-patient dose escalation is permitted if: 1) patient had ≤ Grade 1 drug-related toxicity except alopecia, lymphopenia; 2) Proposed higher dose should have been established as safe; 3) Patients should not have progressive disease on study; 4) Patients on dose level 7 who tolerate (i.e., no grade 2 or greater drug-related adverse events) cycle 1 can escalate to dose level 8 from cycle 2 onwards.

**Phase I MTD Expansion Phase**
Once the MTD is established, up to 15 additional patients will be treated and tumor biopsies will be obtained to assess PD effects. Tumor biopsies will be mandatory during the expansion phase of the study. Biopsies will be obtained at the time points outlined below. Depending on the results obtained from the tumor biopsies in the first few patients, the timing of the biopsy can be adjusted, but the number of tumor biopsies and the procedure will not change. Any change in the timing of the tumor biopsy will be discussed with the patient prior to obtaining informed consent.

For the purposes of final analysis of the expansion phase, only patients with paired tumor biopsies will be considered evaluable. Patients with fewer than the 2 required biopsies will not be considered evaluable for the expansion phase and will need to be replaced in the accrual scheme of the expansion phase.

## 5.1 Agent Administration

Reported adverse events and potential risks are described in Section 7. Appropriate dose modifications are described in Section 6. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

TRC102 will be administered to patients as a capsule dosed according to the dose escalation table. TRC102 should be administered together with TMZ capsules. TRC102/TMZ should be administered either 1 hour before or 2 hours after a meal. Patients should drink an eight ounce glass of water following TRC102/TMZ administration. Antiemetic therapy may be administered prior to and/or following administration of TRC102/TMZ in patients who develop nausea and vomiting but not prophylactically. TRC102/TMZ capsules should not be opened or chewed. If capsules are accidentally opened or damaged, precautions should be taken to avoid inhalation or contact with the skin or mucous membranes.

The drugs will be administered in 28-day cycles (± 1 day for scheduling). A missed or vomited dose will not be replaced. The patients will be instructed to take the next scheduled dose at the regularly scheduled time. Patients will be asked to maintain a Study Medication Diary (Appendix C) and record each dose of medication. Patients will be given instructions for completing the medication diary and will be asked to return it to the clinic staff at the end of each cycle.

## 5.2 Definition of Dose-Limiting Toxicity (Phase I only)

For MTD determination, DLTs will be defined as toxicities occurring within the first cycle of treatment. Patients who come off study for reasons other than drug-related toxicity (e.g., disease progression) prior to completion of more than 90% of dose of the first cycle will be replaced. Dose-limiting toxicity (DLT) is defined as an adverse event that occurs during the first cycle and is felt to be related (possibly, probably, or definitely) to administration of study drugs and fulfills one of the following criteria:

5.2.1 Hematologic Toxicity

5.2.1.1 Grade 4 neutropenia

5.2.1.2 Febrile neutropenia

5.2.1.3 Neutropenic infection: Grade ≥ 3 neutropenia with grade ≥ 3 infection.

5.2.1.4 Grade ≥ 3 thrombocytopenia

5.2.1.5 A drop in Hgb ≥3.0 g/dL over one week

5.2.1.6 Any degree of lymphopenia, or leukopenia in the absence of grade 4 neutropenia will not be considered dose limiting.

5.2.2 Grade ≥ 3 Non-hematologic Toxicity

5.2.2.1 Grade ≥ 3 non-hematological toxicity (except alopecia) felt to be related to study medications will be considered dose-limiting with the following clarifications:

5.2.2.2 Diarrhea Grade 3 will only be considered dose-limiting if it is refractory to treatment as outlined in Section 5.3.2, Supportive Care Guidelines, and unable to be corrected to Grade 2 or less within 24 hours. Bloody or Grade 4 diarrhea will be dose-limiting.

5.2.2.3 Nausea and vomiting Grade 3 will only be considered dose-limiting if it is refractory to anti-emetic therapy and unable to be corrected to Grade 1 or less within 24 hours (Section 5.3.1).

5.2.2.4 Rise in creatinine to Grade 3, not corrected to Grade 1 or less within 48 hours with IV fluids will be considered dose-limiting. All Grade 4 rises in creatinine will be dose limiting.

5.2.2.5 Grade ≥ 3 electrolyte abnormalities will not be considered dose limiting. Grade 4 metabolic toxicities that are symptomatic will be considered dose-limiting regardless of duration or ability to correct.

Management and dose modifications associated with the above adverse events are outlined in Section 6. Dose escalation will proceed within each cohort according to the following scheme.

| Number of Patients with DLT at a Given Dose Level | Escalation Decision Rule |
|---|---|
| 0 out of 3 | Enter 3 patients at the next dose level. |
| ≥ 2 | Dose escalation will be stopped. This dose level will be declared the maximally administered dose (highest dose administered). Three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose. |
| 1 out of 3 | Enter at least 3 more patients at this dose level.<br>• If 0 of these 3 patients experience DLT, proceed to the next dose level.<br>• If 1 or more of this group suffer DLT, then dose escalation is stopped, and this dose is declared the maximally administered dose. Three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose. |
| ≤1 out of 6 at highest dose level below the maximally administered dose | This is generally the recommended phase 2 dose. At least 6 patients must be entered at the recommended phase 2 dose. |

### 5.3 General Concomitant Medication and Supportive Care Guidelines

All patients will be provided with the best available supportive care. All concurrent medications should be documented prior to initiation of treatment, and be periodically reviewed with the patient. Particular attention must be paid to medications which may cause hemolytic anemia.
No other approved or investigational anticancer treatment will be permitted during the study period, including chemotherapy, biologic response modifiers, hormone therapy, immunotherapy, or radiotherapy.

#### 5.3.1 Nausea/Vomiting

Anti-emetics will not be administered routinely prior to TMZ or TRC102. However, if a patient develops nausea/vomiting, anti-emetics such as but not limited to prochlorperazine, metoclopramide, 5-HT3 antagonists, or aprepitant may be given. In addition, if a patient develops nausea and/or vomiting that is Grade 2 or greater, anti-emetics may be instituted prophylactically at the discretion of the investigator. Nausea and vomiting will be considered refractory if it does not resolve to ≤ Grade 1 with treatment with a combination of at least 2 of the antiemetics within 24 hours.

#### 5.3.2 *Diarrhea*

If diarrhea develops and does not have an identifiable cause other than study drug administration, anti-diarrheals such as Lomotil (diphenoxylate HCl 2.5 mg + atropine sulfate 0.025 mg/tablet) dosed according to package insert or loperamide 4 mg po after

the first unformed stool with 2 mg po every 2 hours as long as unformed stools continue (4 mg every 4 hours while asleep). No more than 16 mg of loperamide should be taken in during a 24-hour period. This regimen can be repeated for each diarrheal episode. Diarrhea will be considered refractory if it does not resolve within 24 hours ≤ to Grade 2 with the above regimen (16 mg, or less if there is resolution of the symptoms, of loperamide in a 24-hour period).

5.3.3 *Neutropenia*

Febrile neutropenia is a life-threatening complication requiring hospitalization and urgent broad-spectrum antibiotics, as well as an aggressive search for the source and microbial cause of the episode. Growth factors to prevent neutropenia will not be administered prophylactically. If necessary, they may be administered according to accepted American Society of Clinical Oncology (ASCO) guidelines to allow re-treatment.

5.3.4 *Anemia*

Symptomatic anemia should be treated with red blood cell transfusion and is recommended if the hemoglobin falls below 8 g/dL. Use of erythropoietin will follow ASCO guidelines.

5.3.5 *Thrombocytopeni*a

Thrombocytopenia will be treated conservatively. In the absence of bleeding, or a necessary invasive procedure, platelet transfusions should be given for a platelet count ≤10,000/mm$^3$. If invasive procedure(s) is (are) planned, or the patient develops bleeding, platelet transfusions should be administered in accordance with the standard of practice, usually maintaining a platelet count above 50,000/mm$^3$.

### 5.4 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
- Significant toxicity occurs despite 2 dose reductions as described in Section 6 or no lower dose level exists
- Pregnancy
- 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.

### 5.5 Duration of Follow Up

Patients will be followed for 30 days after the last dose is administered or until one of the following occurs: patient enrolls on another protocol, patient receives standard of care, or death, whichever comes first. The follow-up will consist of a phone call between Days 27-30 after the last dose to evaluate adverse events that were ongoing and any new events that might be deemed related to the therapy. Toxicities felt to be possibly, probably, or definitely related to the study drugs that have not resolved or stabilized by Day 30 post-treatment will be followed until stabilization or resolution via phone calls as clinically indicated. Whenever possible, Phase II patients will be followed via phone calls until they progress or start another treatment for their cancer.

### 5.6 Criteria for Removal from Study

Patients will be removed from study for one of the following reasons: completed 30-day follow-up period or toxicities are unresolved but stabilized. The reason for study removal and the date the patient was removed must be documented in the medical record and communicated to Central Registration per Section 4.

## 6. DOSING DELAYS/DOSE MODIFICATIONS

Non-hematologic toxicities (at least possibly related to therapy) should have resolved to ≤ Grade 1 or baseline prior to starting the next cycle or receiving the next dose of TRC102 or TMZ within a cycle. Grade 2 electrolyte abnormalities, grade 2 nausea/vomiting/diarrhea in the absence of maximal palliative support, or any grade alopecia, will not result in holding or delaying study drugs.

Hematologic toxicities (at least possibly related to therapy) should have resolved to ≤ Grade 1 for thrombocytopenia, ≤ Grade 2 for neutropenia and anemia prior to resuming therapy except any grade lymphopenia, leucopenia in the absence of grade 3 neutropenia.

Even though study drug administration is allowed at these lower grades, every effort should be made to correct the abnormal lab values to normal if possible. If the potassium level is grade 2 or greater and/or if the calcium, magnesium, and/or phosphate are grade 3 or higher, an EKG must be performed and appropriate action taken based on the results.

Treatment may be delayed for a maximum of 2 weeks for toxicities. In case toxicities do not resolve as stated, the patient will not receive further therapy on this protocol and will be followed for resolution of toxicities. Start of next cycle may be delayed for up to 1 week to accommodate scheduling conflicts. Dose modifications are intended for within-cycle and start-of-next-cycle changes.

A maximum of 2 dose reductions will be allowed before patient is taken off treatment. Patients who require a dose reduction will not have the dose re-escalated. For patients who had one dose reduction without significant change in their toxicities (i.e., fatigue), they could be dose reduced to 4 days of the same dose level instead of dropping to the next lower dose level.

Patients will be carefully monitored for evidence of liver toxicity and toxicity will be graded per CTCAE criteria (section 7.2). For patients who develop grade 3 LFT abnormalities, study drugs will be held and the benefits and risks of continuing treatment on a reduced dose will be discussed with the patient by the study team.

### 6.1 Dose Reduction

**Grade 2 Drug-related toxicities**: No changes will be made to the dose of TRC102/TMZ for Grade 2 toxicities.
**Grade 3-4 Drug-related non-hematologic toxicities (except alopecia):** Doses of TRC102/TMZ will be held until toxicities recover to ≤ Grade 1 prior to re-initiating treatment at the lower dose level. Electrolyte abnormalities will not require dose reduction if resolution to Grade 2 or less is documented. Dose modifications for nausea, vomiting, and diarrhea will be made only if they are refractory to treatment (See Section 5.2).
**Grade 3-4 Drug-related thrombocytopenia:** Dose of TRC102/TMZ will be held until it has resolved to ≤ Grade 1 prior to re-initiating treatment at the lower dose level.
**Grade 3-4 Drug-related neutropenia:** Dose of TRC102/TMZ will be held until it has resolved to ≤ Grade 2 prior to re-initiating treatment at the lower dose level.

A drop in Hgb ≥3.0 g/dL over one week that results in grade 3 anemia: Dose of TRC102/TMZ will be held until it has resolved to ≤ Grade 2 prior to re-initiating treatment at the lower dose level.
Any grade lymphopenia, leucopenia in the absence of at least grade 3 neutropenia**:** Dose of TRC102/TMZ will not be held or modified.

**Definition of Complete Course:** A cycle is defined as 28 days, with D1 starting the first day of TRC102/TMZ administration. If a patient receives 90% of scheduled treatments of TRC102/TMZ and remains in the study until D28, the patient will be considered to have completed a cycle of therapy. Patients who do not complete a cycle of therapy for reasons other than toxicity will be replaced. Patients who do not complete one cycle of therapy due to toxicity will not be replaced. All patients should have completed one cycle of therapy (unless removed from the study due to toxicity), at which point they will be evaluable.

## 7. 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 7.1) and the characteristics of an observed AE (Section 7.2) will determine whether the event requires expedited reporting (via CTEP-AERS) **in addition** to routine reporting.

### 7.1 Comprehensive Adverse Events and Potential Risks List(s) (CAEPRs)

**Comprehensive Adverse Events and Potential Risks list (CAEPR) for Methoxyamine hydrochloride (TRC102, NSC 3801)**

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 with bold and 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.
The CAEPR does not provide frequency data; refer to the Investigator's Brochure for this information. Below is the CAEPR for Methoxyamine hydrochloride (TRC102).

**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 1.1, April 25, 2019[1]**

| **Adverse Events with Possible Relationship to Methoxyamine hydrochloride (TRC102) (CTCAE 5.0 Term)** | **Specific Protocol Exceptions to Expedited Reporting (SPEER)** |
|---|---|
| BLOOD AND LYMPHATIC SYSTEM DISORDERS | |
| Anemia | ***Anemia (Gr 2)*** |
| GASTROINTESTINAL DISORDERS | |
| Diarrhea | ***Diarrhea (Gr 1)*** |
| Mucositis oral | ***Mucositis oral (Gr 1)*** |
| Nausea | ***Nausea (Gr 2)*** |
| Vomiting | ***Vomiting (Gr 2)*** |
| GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS | |
| Fatigue | ***Fatigue (Gr 2)*** |
| Fever | ***Fever (Gr 1)*** |
| INVESTIGATIONS | |
| Blood bilirubin increased | ***Blood bilirubin increased (Gr 1)*** |
| Haptoglobin decreased | ***Haptoglobin decreased (Gr 1)*** |
| METABOLISM AND NUTRITION DISORDERS | |
| Anorexia | ***Anorexia (Gr 1)*** |
| SKIN AND SUBCUTANEOUS TISSUE DISORDERS | |
| Pruritus | ***Pruritus (Gr 1)*** |
| Rash maculo-papular | ***Rash maculo-papular (Gr 1)*** |

[1]This 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.

**Adverse events reported on Methoxyamine hydrochloride (TRC102) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that Methoxyamine hydrochloride (TRC102) caused the adverse event:**

**BLOOD AND LYMPHATIC SYSTEM DISORDERS** - Hemolysis
**GASTROINTESTINAL DISORDERS** - Constipation
**IMMUNE SYSTEM DISORDERS** - Allergic reaction
**INVESTIGATIONS** - Alanine aminotransferase increased; Aspartate aminotransferase increased; Creatinine increased; Lymphocyte count decreased; Neutrophil count decreased; Platelet count decreased; White blood cell decreased
**METABOLISM AND NUTRITION DISORDERS** - Hypocalcemia
**NERVOUS SYSTEM DISORDERS** - Dysgeusia
**PSYCHIATRIC DISORDERS** - Psychosis
**RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS** - Dyspnea
**VASCULAR DISORDERS** - Thromboembolic event

**Animal Data: The following toxicities have been observed in animal studies with Methoxyamine hydrochloride (TRC102):**

**Dogs**
**BLOOD AND LYMPHATIC SYSTEM DISORDERS** - Bone marrow hypercellularity; Epididymal cellular debris; Small thymus
**GASTROINTESTINAL DISORDERS** - Abnormal excreta
**INVESTIGATIONS** - Increased reticulocytes, Lymphocytes increased
**NERVOUS SYSTEM DISORDERS** - Convulsions; Tremors
**REPRODUCTIVE SYSTEM AND BREAST DISORDERS** - Seminiferous tubule degeneration

**Rats**
**BLOOD AND LYMPHATIC SYSTEM DISORDERS** - Enlarged or Swollen spleens; Increased spleen weight
**INVESTIGATIONS** - Increased monocytes; Increased reticulocytes; Lymphocytes increased

**Note**: Methoxyamine hydrochloride (TRC102) 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.

### 7.1.1 Adverse Event Lists for TMZ

The most common adverse reactions (≥10% incidence) are alopecia, fatigue, nausea, vomiting, headache, constipation, anorexia, convulsions, rash, hemiparesis, diarrhea, asthenia, fever, dizziness, coordination abnormal, viral infection, amnesia, and insomnia. The most common Grade 3 to 4 hematologic laboratory abnormalities (≥10% incidence) that have developed during treatment with temozolomide are lymphopenia, thrombocytopenia, neutropenia, and leucopenia which requires monitoring absolute neutrophil count (ANC) and platelet count prior to dosing and throughout treatment. Cases of myelodysplastic syndrome and secondary malignancies, including myeloid leukemia, have been observed. All patients, particularly those receiving steroids, should be observed closely for the development of lymphopenia and PCP.
Cases of hepatic injury, including fatal hepatic failure, have also been observed in patients enrolled on temozolomide studies; it was noted that liver toxicity may occur

several weeks or more after initiation of treatment or after temozolomide discontinuation.
Refer to the package insert for the comprehensive list of adverse events.

## 7.2 CTEP Reporting Requirements

### 7.2.1 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 4.0 will be utilized until March 31, 2018 for AE reporting. CTCAE version 5.0 will be utilized for AE reporting beginning April 1, 2018. 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 web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **'Expectedness'**: AEs can be 'Unexpected' or 'Expected' (see Section 7.1 above) for expedited reporting purposes only. 'Expected' AEs (the ASAEL) are ***bold and italicized*** in the CAEPR (Section 7.1).

- **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.

### 7.2.2 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions. **AEs reported through CTEP-AERS must <u>also</u> be reported in routine study data submissions.**

### 7.2.3 Expedited Adverse Event Reporting

7.2.3.1 Expedited AE reporting for this study must use CTEP-AERS (CTEP Adverse Event Reporting System), accessed via the CTEP Web site (http://ctep.cancer.gov). The reporting procedures to be followed are presented in the "NCI Guidelines for Investigators: Adverse Event Reporting Requirements for DCTD (CTEP and CIP) and DCP INDs and IDEs" which can be downloaded from the CTEP Web site (http://ctep.cancer.gov). These requirements are briefly outlined in the tables below.

In the rare occurrence when 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 phoned in must be entered electronically into CTEP-AERS by the original submitter at the site.

7.2.3.2 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, unless as noted below. 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.

**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** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)

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

1) Death
2) A life-threatening adverse event
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

**ALL SERIOUS** adverse events that meet the above criteria MUST be immediately reported to the NCI via CTEP-AERS within the timeframes detailed in the table below.

| Hospitalization | Grade 1 and Grade 2 Timeframes | Grade 3-5 Timeframes |
|---|---|---|
| Resulting in Hospitalization ≥ 24 hrs | 10 Calendar Days | 24-Hour 5 Calendar Days |
| Not resulting in Hospitalization ≥ 24 hrs | Not required | |

**NOTE:** Protocol specific exceptions to expedited reporting of serious adverse events are found in the Specific Protocol Exceptions to Expedited Reporting (SPEER) portion of the CAEPR.

**Expedited AE reporting timelines are defined as:**

- "24-Hour; 5 Calendar Days" - The AE must initially be reported via CTEP-AERS within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.
- "10 Calendar Days" - A complete expedited report on the AE must be submitted within 10 calendar days of learning of the AE.

[1]Serious adverse events 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 notification followed by complete report within 5 calendar days for:**

- All Grade 3, 4, and Grade 5 AEs

**Expedited 10 calendar day reports for:**

- Grade 2 AEs resulting in hospitalization or prolongation of hospitalization

[2]For studies using PET or SPECT IND agents, the AE 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: May 5, 2011

7.2.3.3 Protocol-specific expedited AE reporting exclusions

For this protocol only, certain AEs/grades are exceptions to the Expedited Reporting Guidelines and do not require expedited reporting (i.e., CTEP-AERS). These are: any grade lymphopenia, any grade alopecia, Grade 2 electrolyte (sodium, potassium, phosphorous, magnesium) abnormalities, Grade 2 anemia, Grade 2 hypoalbuminemia, Grade 2 hyperglycemia, Grade 2 INR, Grade 2 PTT, and Grade 2 hyperuricemia will NOT be reported through CTEP-AERS but will be reported in the routine data submissions.

7.2.3.4 Pregnancy, Fetal Death, and Death Neonatal

NOTE: When submitting CTEP-AERS reports for "Pregnancy", "Pregnancy loss", or "Neonatal loss", the Pregnancy Information Form should be completed and faxed along with any additional medical information to 301-230-0159. The potential risk of exposure of the fetus to the investigational agent(s) or chemotherapy agent(s) should be documented in the "Description of Event" section of the CTEP-AERS report.

7.2.3.5 Pregnancy

- Because patients who become pregnant on study risk intrauterine exposure of the fetus to agents which may be teratogenic, DCTD/DCP is requesting that pregnancy should be reported in an expedited manner via CTEP-AERS as Grade 3 "Pregnancy, puerperium and perinatal conditions - Other (pregnancy)" under the Pregnancy, puerperium and perinatal conditions SOC.
- The pregnancy outcome for patients on study should be reported via CTEP-AERS at the time the outcome becomes known, accompanied by the same Pregnancy Report Form used for the initial report.

7.2.3.6 Pregnancy loss

- Pregnancy loss is define in CTCAE as "Death in utero."
- Any pregnancy loss should be reported expeditiously, as Grade 4 "Pregnancy loss" under the Pregnancy, puerperium and perinatal conditions SOC.
- A pregnancy loss should NOT be reported as a Grade 5 event under the Pregnancy, puerperium and perinatal conditions SOC, as currently CTEP-AERS recognizes this event as a patient death.

7.2.3.7 Death Neonatal

- Neonatal death, defined in CTCAE as "A disorder characterized by cessation of life occurring during the first 28 days of life" that is felt by the investigator to be at least possibly due to the investigational agent/intervention, should be reported expeditiously.
- A neonatal death should be reported expeditiously as Grade 4 "Death neonatal" under the General disorders and administration SOC.
- Neonatal death should NOT be reported as Grade 5 "Death neonatal" under the General disorders and administration SOC. If reported as such, the CTEP-AERS interprets this as a death of the patient being treated.

7.2.4 Information regarding transfusion requirements in the first 3 cycles of treatment will be reported to CTEP. It will not require expedited reporting.

7.2.5 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 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.

7.2.6 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 reporting via CDUS unless otherwise specified.

## 7.3 NIH Reporting Requirements

### 7.3.1 Definitions

Please refer to definitions provided in Policy 801: Reporting Research Events (https://irbo.nih.gov/confluence/display/IRBO/Policies+and+SOPs).

### 7.3.2 OHSRP Office of Compliance and Training / IRB Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found at https://irbo.nih.gov/confluence/display/IRBO/Policies+and+SOPs

Note: Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported per these policies.

### 7.3.3 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found at
https://irbo.nih.gov/confluence/display/IRBO/Policies+and+SOPs

### 7.3.4 NCI Clinical Director Reporting

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

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

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

# 8. PHARMACEUTICAL INFORMATION

## 8.1 TRC102 (NSC 3801)

**Chemical Name:** Methoxyamine hydrochloride

**Other Names:** Methoxylamine HCl

**Classification:** Biochemical inhibitor of the BER pathway

**Molecular Formula:** $CH_5NO•HCl$ **M.W.:** 83.52 Daltons

**Approximate Solubility:** At ambient temperature, TRC102 is freely soluble in water, sparingly in ethanol (70 mg/mL), and slightly in DMSO (140 mg/mL).

**Mode of Action:** TRC102 has the ability to interrupt the process of base excision repair (BER) by binding to apurinic/apyrimidinic sites produced during the initial step of the BER pathway. These sites are substrates for topoisomerase II (topo II); an enzyme that cleaves damaged DNA. TRC102 has demonstrated the ability to potentiate the activity of the alkylating agents temozolomide and carmustine, and antimetabolite agents fludarabine and pemetrexed, in murine models of human cancer. Therefore, TRC102 may be able to potentiate the activity of alkylating and antimetabolite chemotherapy in patients.

**Description:** TRC102, a white, crystalline solid, is the hydrochloride salt of methoxyamine.

**How Supplied:** TRC102 is supplied by IriSys, Inc. (white capsules) or the Developmental Therapeutics Program, DCTD/NCI (pink capsules) and distributed by the Pharmaceutical Management Branch, CTEP/DCTD/NCI. Each opaque, size 2, hard gelatin capsules contain 25 mg of Methoxyamine HCl powder, microcrystalline cellulose, crospovidone, sodium starch glycolate, colloidal silicon dioxide, and talc. Each HDPE bottle has a child-resistant screw cap and contains 30 capsules.

**Storage:** Store bottles of TRC102 at refrigerated temperature (2-8°C).

**Stability:** Shelf life surveillance of the intact bottles in ongoing.

**Route of Administration:** Oral

**Method of Administration:** Capsules are taken in the morning after patients have fasted for at least two hours. Take with 8 ounces of water. Capsules should be swallowed whole, do not chew or crush. Patients should refrain from eating or drinking for one hour following TRC102 dosing.

**Elimination:** There have been no data published on excretion or metabolism of TRC102.

Based on the chemical structure of such a small molecule, no CYP interaction is expected

### 8.2 Temozolomide

Temozolomide is commercially available. Please refer to the FDA-approved package insert for more information about this drug. Full prescribing information is available at: http://www.temodar.com/temodar/index.do

Chemical Name: 3,4-dihydro-3-11 methyl-4-oxoimidazo[5,1-d]-as-tetrazine-8-carboxamide.

Other Names: Temodar®, Temodal, Temcad

Molecular Formula: $C_6H_6N_6O_2$ (M.W: 194.15)

Mode of Action: Temozolomide is not directly active but undergoes rapid nonenzymatic conversion at physiologic pH to the reactive compound MTIC. The cytotoxicity of MTIC is thought to be primarily due to alkylation of DNA. Alkylation (methylation) occurs mainly at the O6 and N7 positions of guanine.

How Supplied: Capsules are supplied in amber glass bottles with child resistant polypropylene caps containing the following capsule strengths: 5 mg; 20 mg; 100 mg; 140 mg; 180 mg; 250 mg. The dose of temozolomide is based on BSA then rounded off to the nearest 5 mg.

Storage: Store at 25°C (77°F); excursions permitted to 15°-30°C (59°-86°F).

Stability: The molecule is stable at acidic pH (<5), and labile at pH >7

Route of Administration: Oral: Take each day's dose of capsules at one time, with a full glass of water. They should be swallowed whole and never chewed. If capsules are vomited do not take a second dose.

Potential Drug Interaction: Administration of valproic acid decreases oral clearance of temozolomide by about 5%.

Availability: Temozolomide is commercially available

### 8.3 Agent Ordering and Agent Accountability

8.3.1 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.
Submit agent requests through the PMB Online Agent Order Processing (OAOP) application. Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account and the maintenance of an "active" account status, a "current" password, and an active registration status. For questions about drug orders, transfers, returns, or accountability, call or email PMB any time. Refer to the PMB's website for specific policies and guidelines related to agent management.

8.3.2 Agent Inventory Records – The investigator, or a responsible party designated by the investigator, must maintain a careful record 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.

8.3.3 Investigator Brochure Availability

The current versions of the IBs for the agents will be accessible to site investigators and research staff through the PMB OAOP application. Access to OAOP requires the establishment of a CTEP IAM account and the maintenance of an "active" account status, a "current" password, and an active registration status. Questions about IB access may be directed to the PMB IB Coordinator via email.

8.3.4 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 Online Agent Order Processing (OAOP) application: https://ctepcore.nci.nih.gov/OAOP
- 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)

## 9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

### 9.1 Pharmacokinetics (Phase I only)

Blood samples for PK analyses (optional) will be collected prior to drug administration and 1, 2, 3, 4, 8, 12, and 24 hours post-dosing on cycle 1 day 1 in both the escalation and expansion phases and on cycle 1 day 5, prior to dosing on that day in the expansion phase only. Based on results from initial measurements, sampling times may be adjusted, but neither the total number of samples nor the total amount of blood drawn per patient will be increased. Samples will be collected in lavender-top (EDTA) tubes with 2 mL of blood per sample and stored refrigerated (2-8°C). All samples will be centrifuged and plasma will be stored at -70°C for analysis as described in Appendix E. Samples will be analyzed using a validated LC-MS or LC-MS/MS method in human plasma.

Urine (optional) will be collected prior to drug administration on cycle 1 day 1, and kept separate and then treat as described in Appendix E. Urine will then be collected at every void from 0 to 24 hours post-treatment on day 1 in cycle 1 for PK analysis. Start urine collection immediately after study drug is administered and store refrigerated (2-8°C) acidified as described in Appendix E.

Send PK samples to the following lab contact:

Laboratory Contact:
Tracy W. Webb
Office of the Associate Director/DTP/NCI
FNLCR
Boyles Street
Building 1047, Room 8
Frederick, MD 21702
301-846-7402; webbtw@mail.nih.gov

### 9.2 Pharmacodynamics

Tumor tissue: Evaluation of drug effect on DNA damage response will be performed by immunofluorescence assay for measurement of γH2AX levels and other biomarkers of DNA damage response/repair. Epithelial-mesenchymal transition (EMT), MGMT, and levels of cCasp3 (apoptosis) will also be measured. Any remaining tumor tissue may be formalin-fixed and paraffin-embedded and sent to the laboratory of Dr. Stanton Gerson (Case Western Reserve University) for IHC assessment of the enzyme N-methylpurine DNA glycosylase (MPG). No patient data will accompany any samples sent to Dr. Gerson's lab.

#### 9.2.1 Laboratory Contact

At least 24 hours prior to tumor biopsy or blood sample collection, the research nurse will contact the NCI Phase I/II PK/PD Support Group in NIH Building 10:
E-mail (preferred): NCIPK-PDsupportgroup@mail.nih.gov

Pager (preferred): 102-12798
Phone: 240-858-3963
Fax: 301-480-5871.

For biopsies, tubes pre-labeled with the information specified in Section 9.2.4, biopsy date, and site of tissue biopsy will be provided. Initial processing and shipping of the samples will be completed as described below. If a patient had a biopsy within the past 3 months and had no treatment since that biopsy, an attempt will be made to obtain and process that tissue as the baseline biopsy.

9.2.2 Blood Collection for CTC Studies (Optional)

At each time point, whole blood (at least 7.5 mL) will be collected aseptically by venipuncture or from a venous port into one 10 mL Streck tube (catalog number 218962). One 10 mL RareCyte tube (catalog number 24-1070-005) is also acceptable. Tubes must be inverted 8 times to ensure adequate mixing of the additive. Blood samples for CTCs will be collected from patients at the following time points:

Phase I

- Cycle 1 prior to treatment
- Cycle 1, day 1: 8 hrs after drug in both the escalation and expansion phases
- Cycle 1, day 5 (±1 day in case of scheduling difficulties): before drug in the expansion phase only
- Cycle 2, day 1: before drug in both the escalation and expansion phases
- Day 1 of all subsequent cycles: before drug in both the escalation and expansion phases
- At time of disease progression

Phase II

- Cycle 1 prior to treatment
- Cycle 1 day 4 or 5 before drug (on the same day as the post-dose biopsy)
- Day 1 (±1 day) of all subsequent cycles before drug
- At time of disease progression

Blood for CTC analysis will be shipped to the PADIS laboratory on the day it is collected. Arrangement will be made for pickup with the CSP courier service (301-846-5893) and the receiving laboratory will be notified by email of the shipment (NCIPDSupportPADIS@mail.nih.gov). Because of the 48-hour window of CTC sample stability, no CTC specimens should be collected or shipped on Friday afternoon.

**Samples will be sent to:**
Attn: PADIS CTC Laboratory
Frederick National Laboratory for Cancer Research
Leidos Biomedical Research, Inc.
1050 Boyles Street
Building 425, Room 102
Frederick, MD 21702

Phone: 301.846.4711
NCIPDSupportPADIS@mail.nih.gov

9.2.2.1 Measurement of Circulating Tumor Cells (CTC) in Peripheral Blood

PADIS, Frederick National Lab, is developing and validating the process to isolate circulating tumor cells using the ApoStream™ technology platform which uses size and cell surface charge to isolate cancer cells; this platform is of particular interest in that it can detect CTCs from patients with cancer of mesenchymal origin, such as sarcomas.

9.2.3 Tumor Biopsies

9.2.3.1 Timing of tumor biopsies

Biopsies will be optional during the escalation phase and mandatory in up to 15 additional patients enrolled at the MTD during the Phase I expansion phase and during the Phase II portion of the study (patients with NSCLC will not be biopsied). Biopsies will be collected:

- before drug administration on study (baseline), and
- post-treatment:
  - Phase I: on cycle 1, day 5 (± 1 day in case of scheduling difficulties), 3-4 hrs after drug
  - Phase II: on cycle 1, day 4 or 5, 3-4 hrs after drug
- OPTIONAL—One optional tumor biopsy may be collected:
  - A restaging follow-up biopsy may be performed on day 1 (± 2 days) of the cycle following any restaging at which a 10-19% increase in tumor volume is observed (according to RECIST criteria) if the patient has been on study for at least 4 cycles

  OR
  - A progression tumor biopsy may be performed at the time of disease progression

9.2.3.2 Biopsy Procedure

Serial tumor biopsies will be obtained by the Interventional Radiology team by a percutaneous approach, a dermatologist for skin lesions, or an ENT for lesions that are easily biopsiable through ENT exam. If the patient has skin lesions or peripheral lymph nodes that are easily accessible by the dermatologist or surgeon and could be done under local anesthesia with < 5% risk of complications, this will be acceptable if agreeable with the patient and investigator. If considered feasible and safe by the biopsy team, up to five core biopsies ≥18 gauge in diameter and ≥1 cm in length, or equivalent, will be obtained during each procedure. It is estimated that there will be between 2 million–5 million cells from each biopsy. If a site is deemed appropriate for biopsy with minimal risk to the participant by agreement between the investigators and the biopsy team, an attempt for biopsy will be made. If possible, the lesion from which each biopsy is taken will be documented.

The use of imaging to facilitate biopsies will be decided by members of the biopsy team and may include ultrasound, CT scan, or MRI. Should a CT scan be needed for biopsy, the number of scans for each procedure will be limited to the minimum number needed to safely obtain a biopsy. Tumor biopsies and local anesthesia will be administered only if they are considered to be of low risk to the participant, as determined by the investigators and the biopsy team. In the expansion cohort, if the participant chooses not to undergo tumor biopsy after the initial attempt, he/she will still remain in the study and receive study medication, and all the other correlative studies will be performed.

Tumor biopsies are optional during the escalation phase but mandatory for the 15 additional patients enrolled at the MTD during the expansion phase and Phase II portion of the study. Baseline biopsies will be performed following patient enrolling on study or if the patient had a biopsy within the past 3 months and had no treatment since that biopsy, an attempt will be made to obtain and process that tissue as baseline biopsy. If the tissue could not be used for planned PD studies, a biopsy will be performed. If an initial attempt at biopsy is unsuccessful, the patient will be given an option to proceed with a repeated attempt. A separate consent form must be signed for each biopsy procedure, so patients may choose not to undergo subsequent biopsies. If the baseline biopsy is unsuccessful or the patient refuses to undergo subsequent biopsies, no further biopsies will be performed but the patient will remain on study, receive study medication, and other correlative studies will be performed. The patient will be replaced in the accrual scheme of the expansion phase.

9.2.3.3 Solid Tumor Biopsy Processing

Up to five tissue cores, or equivalent, will be collected and flash frozen in liquid nitrogen and submitted for evaluation of DNA damage markers, and H&E pathology evaluation. The frozen biopsy specimens are transferred to PADIS, where the biopsy samples are stored at -80°C until processing. Refer to SOP340507 (https://dctd.cancer.gov/ResearchResources/biomarkers/docs/par/SOP340507_Biopsy_Frozen.pdf) for detailed instructions on biopsy collection and handling. The sample shipping manifest and batch record are included in SOP340507. Biopsy samples will be analyzed for PD and genomic analyses based on the assay prioritization shown in Figure 4 and the discretion of the PI.

Biopsies will be shipped on dry ice to:
ATTN: PADIS IQC Lab
Frederick National Laboratory for Cancer Research
Leidos Biomedical Research, Inc.
1050 Boyles Street
Building 425, Room 105
Frederick, MD 21702
Phone: 301.846.1951 or 301.846.6747
NCIPDSupportPADIS@mail.nih.gov

Shipment should be by CSP Courier and may be arranged by contacting Mike Johnston, FNLCR, Tel.: 301-846-5893

*Note: Per the discretion of the PI, restaging follow-up or progression biopsies may be collected per SOP to be used for analyses other than those listed here, including analyses carried out as a part of other DTC clinical trials.*

9.2.3.4 Archival Tissue Submission

Archival tumor tissue submitted as a baseline specimen must have been collected within 3 months prior to patient registration, and the patient must not have received any intervening cancer therapy since collection of the specimen. Archival tissue must be collected and processed according to SOP340507 (https://dctd.cancer.gov/ResearchResources/biomarkers/docs/par/SOP340507_Biopsy_Frozen.pdf), including flash-freezing in liquid nitrogen, minimal cold ischemia time (< 5 minutes), and shipment on dry ice.

Please send an email to FNLCR PD Specimen Central Receiving (NCIPDSupportPADIS@mail.nih.gov_) to advise that archival tissue is being prepared for shipment. State "Protocol Name PD Specimens Ready for Shipment" in the subject line. If needed, FNLCR PD Central Receiving can be contacted directly at 301-846-1951 or 301-846-6747.

## 9.3 Exploratory Genomic Analyses

As of **Amendment R** (5/12/2018), the MoCha laboratory will investigate the occurrence of tumor genomic alterations potentially associated with sensitivity and/or the development of resistance to TRC102 and temozolomide, such as the status of mismatch repair genes or the development of increased mutational load and neoantigen production. Whole exome (WES) and whole transcriptome (RNASeq) genomic analysis of tumor samples collected before and during treatment and upon progression will be performed and compared with data from germline tissue to identify somatic variants, which will be used to determine tumor mutational load and identify neoantigens.

### 9.3.1 **Tumor Genomic Analyses**

Tumor biopsies will be collected and processed as detailed in Section 9.2.3.3.

#### 9.3.1.1 **Baseline and Cycle 1, Day 4/5 Biopsies**

Paired tumor biopsy tissues collected at baseline and after treatment on cycle 1, day 4 or 5 will be used for exploratory targeted (OCAv3) and WES analyses only if there is sufficient tissue remaining after PD biomarker analyses have been completed (Figure 4). Tissue availability will be tracked in real time using Labmatrix. Although the MoCha Laboratory is CLIA-certified, these sequencing studies will not be done per CLIA specifications as these data will not be returned to the patients or used for clinical decision making.

9.3.1.2 **Restaging Follow-Up and Progression Biopsies**
Tumor tissue collected at the (optional) restaging follow-up or progression biopsy will be analyzed by the CLIA-certified OCAv3 assay and results will be returned to patients in the form of the Oncomine report. If additional tumor tissue is available after the OCAv3 analysis, exploratory, non-CLIA certified genomic analyses such as WES may be conducted but the results will not be returned to patients or used for clinical decision making (Figure 4). *Note: Per the discretion of the PI, restaging follow-up or progression biopsies may be used for other analyses, including analyses carried out as a part of other DTC clinical trials, providing that the tissue is collected per SOP for those analyses.*

9.3.2 **Germline Genomic Analysis**
One whole blood sample (10-mL Streck tube) will be collected before drug administration on cycle 1 day 1 (or another time while the patient is on study) and mononuclear cells isolated for nucleic acid extraction and exploratory WES and RNAseq to compare somatic variant calls, mutational load, MMR inactivation, and other potential markers of drug sensitivity or resistance to the tumor biopsy specimen, the results of which will not be returned to patients (Figure 4).

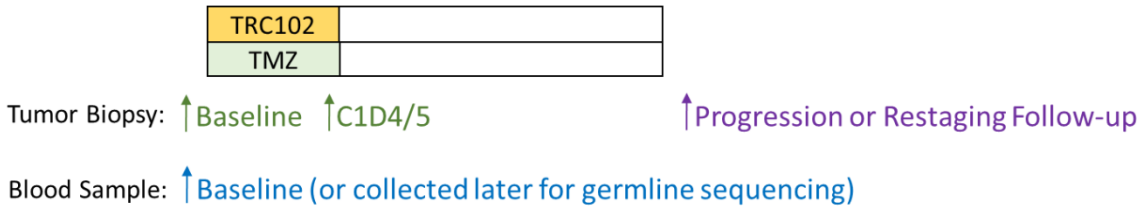
Blood specimens for germline genomic analysis will be shipped at ambient temperature to:

ATTN: Gloryvee Rivera/Lindsay Dutko
MoCha Histology Lab
Frederick National Laboratory for Cancer Research
Leidos Biomedical Research, Inc.
1050 Boyles Street
Building 321 Room 107
Frederick, MD 21702

*Shipment Notification:*
MoChaSampleReceiving@nih.gov

Samples should be labeled with only the unique patient ID. **Do NOT include patient identifiers (e.g., medical record number, patient name, or initials) with the samples.**

**Fig. 4. Summary of Planned Tumor and Germline Genomic Analyses:**




| | Baseline and C1D4/5 Tumor Biopsies | Progression or Restaging Follow-up Biopsy | Blood Sample for Germline Sequencing |
|---|---|---|---|
| Collection Information | All biopsy tissues flash frozen | All biopsy tissues flash frozen | 1 Streck tube |
| First Priority Assay(s) | PD assays | CLIA OCAv3 assay > non-CLIA WES and RNAseq | WES and RNAseq |
| Second Priority Assay(s) | WES and RNAseq | PD assays | |
| Reporting to Patients | None | Oncomine report from OCAv3 assay | Incidental findings only |

9.3.3 **Privacy Considerations**

As patient clinical response data (both for this study and, potentially, after a patient is off-study) will be required for comparison to sequencing results, de-identifying the samples is not feasible. A Certificate of Confidentiality has been obtained to help protect the privacy of all study participants. The informed consent document for this protocol after **Amendment R** (5/12/2018) contains language informing patients about the performance of genetic studies, and patients will have the option to choose whether they wish to take part in these studies.

9.3.4 **Management of Results and Genetic Counseling**

Whole-exome sequencing (WES) of tumor and blood performed for research purposes can detect non-ambiguous germline variants, which may raise health and privacy implications for the patient and his or her family. WES will not be validated for clinical use, and no clinical decisions can be made based on its results.

The results of the CLIA-certified OCAv3 targeted sequencing assay on restaging follow-up or progression tumor biopsy tissue at MoCha (CLIA certification #21D2097127) will be returned to patients in the Oncomine report.

### 9.4 Sample Collection and Processing

Biospecimens will be collected and processed using validated SOPs that will ensure both specimen quality and patient confidentiality pursuant to informed consent provisions. Information about each specimen (e.g., blood, tumor biopsy, circulating tumor cells, per specific protocol) will be recorded on a PK/PD collection worksheet included in Appendix D.

Using a computerized inventory system and a backup hardcopy process, all specimen collection and processing steps will be documented and the specific location of each specimen will be tracked. Each new specimen collected will be assigned a unique barcode identifier that can be linked to the original specimen collected and other relevant information within the inventory system. To ensure patient confidentiality, only containers used for the initial specimen collections will be labeled with patient identifiers.

Only the barcode identifier will be applied to all subsequent specimen containers. When specimens are processed and aliquoted, no patient information will be included on the new containers. Original specimen containers will be discarded. Only barcode-labeled specimens without patient identifiers will be shipped for analysis and/or storage. Specimen labels will indicate: CTEP protocol number, unique patient accession number, 3-digit sample number (see list below), collection time, and total volume collected, as appropriate. Samples from sets of at least three patients will be grouped for scientific analysis.

Standardized 3-digit sample collection numbers:
000 series: blood for germline WES
100 series: urine for PK
200 series: blood for PK
300 series: blood for PD
400 series: blood for circulating tumor cells (CTCs)
500 series: tumor biopsies

The inventory process contains other security provisions sufficient to safeguard patient privacy and confidentiality. Access to the inventory system and associated documents will be restricted to appropriate individuals. Requests to use specimens stored in the repository must be approved. The only patient information available in the inventory system will be the patient sex, diagnosis, and level of informed consent given. SOPs ensure that any changes in informed consent made by a patient and relayed to the PI will be reflected in the inventory system to ensure that specimens are destroyed as appropriate. All laboratory personnel will be trained to adhere to SOPs and will be monitored for high-quality performance.

Any new use of these samples will require prospective IRB review and approval. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material.

If at any time, a patient withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed (or returned to the patient, if so requested), and reported as such to the IRB. Any samples lost (in transit or by a researcher) or destroyed due to unknown sample integrity (i.e., broken freezer allows for extensive sample thawing, etc.) will be reported as such to the IRB.

9.4.1 Human Data Sharing Plan

*What data will be shared?*

We will share human data generated in this research for future research as follows:
X De-identified data in an NIH-funded or approved public repository
X Identified data in BTRIS (automatic for activities in the Clinical Center)
X De-identified or identified data with approved outside collaborators under appropriate agreements

*How and where will the data be shared?*

Data will be shared through:
X An NIH-funded or approved public repository: clinicaltrials.gov
X BTRIS (automatic for activities in the Clinical Center)
X Approved outside collaborators under appropriate individual agreements
X Publication and/or public presentations

*When will the data be shared?*

X At the time of publication or shortly thereafter

## 10. STUDY CALENDAR

Baseline evaluations are to be conducted within 8 days 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 cycle of therapy. Start of next cycle may be delayed for up to 1 week to accommodate scheduling conflicts. History and physical examination and laboratory evaluations can be performed up to 1 week before the start of the next cycle.

| | **Pre-Study** | **C1 W1** | **C1 W2[k]** | **C1 W3[k]** | **C1 W4[k]** | **C2 W1[k]** | **C2 W3** | **C3 W1[k]** | **C3 W3[k] on** | **Off Treatment[j]** |
|---|---|---|---|---|---|---|---|---|---|---|
| TRC102/TMZ[a] | | X | | | | X | | X | | |
| Informed consent | X | | | | | | | | | |
| Demographics | X | | | | | | | | | |
| Medical history | X | | | | | X | | X | | |
| Concurrent meds | X | X---------- | | | | | | | | X |
| Physical exam[b] | X | X | | | | X | | X | | X |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Vital signs | X | | | | | X | | X | | X |
| Height | X | | | | | | | | | |
| Weight | X | | | | | X | | X | | X |
| Performance status | X | | | | | X | | X | | X |
| CBC w/diff, plts[c] | X | | X | X | | X | | X | | |
| Serum chemistry[c] | X | | X | X | | X | | X | | |
| Anemia[l] | X | X | X | | | | | | | |
| PT, INR, PTT[d] | X | | | | | | | | | |
| β-HCG[e] | X | | | | | | | | | |
| AE evaluation | | X----------X | | | | | | | | X |
| Tumor measurements | X | Tumor measurements are repeated every 2 cycles (3 cycles for patients on study more than 1 year). Documentation (radiologic) must be provided for patients removed from study for progressive disease. | | | | | | | | |
| Tumor biopsy[f] | X | X | | | | | | | | |
| PK blood[g] | | X | | | | | | | | |
| Circulating tumor cells[h] | | X | | | | X | | | | X |
| ECG[i] | X | | | | | | | | | |

a: TRC102 and TMZ will be administered once daily, days 1-5, in 28 day cycles.
b: Physical exam at the Clinical Center should be performed at the start of each cycle (up to 1 week before start of a new cycle). Start of the next cycle may be delayed for up to 1 week to accommodate scheduling conflicts.
c: Serum chemistry (albumin, total bilirubin, calcium, creatinine, phosphorus, magnesium, potassium, sodium, SGOT [AST], SGPT [ALT], LDH); CBC w/diff, platelets up to week 3 during cycle 1 (Phase I only), and during week 1 in cycle 2 and subsequent cycles (up to 1 week before start of a new cycle) (Phase I and II). If clinically indicated, labs may be obtained more frequently.
d. PT/INR, PTT as clinically indicated.
e: Serum pregnancy test (women of childbearing potential) within 8 days prior to enrollment and as clinically indicated.
f: Tumor biopsies (mandatory only in Phase I expansion cohort and Phase II where appropriate) will be performed in patients who have disease amenable to biopsy prior to treatment and 3-4 hours after the 5th dose of TRC102 on C1D5 (±1 day in case of scheduling difficulties; day 4 or 5, 3-4 hrs after drug for patients on Phase II reflecting likely start day/weekend timing). One optional biopsy at progression or on day 1 (± 2 days) of the cycle following any restaging at which a 10-19% increase in tumor volume is observed (according to RECIST criteria) for patients who have been on study for at least 4 cycles may also be obtained.
g: Phase I only: PK samples (optional) will be performed at the following time points: C1D1: Pre-dose, at 1, 2, 3, 4, 8, 12, and 24 hours after the first dose in both the escalation and expansion phases; C1D5 pre-dose in the expansion phase only. Additional PK sampling will be optional (but encouraged).
h: Circulating tumor cells (optional) will be collected as described in Section 9.2
i: ECG will be performed at baseline for evaluation of QTc and as clinically indicated thereafter.
j: Off-study evaluation.
k: ±1 day in case of scheduling difficulties

| l: refer to Appendix F |
|---|

## 11. MEASUREMENT OF EFFECT

### 11.1 Antitumor Effect – Solid Tumors

Although response is not the primary endpoint of this trial, patients with measurable disease will be assessed by standard criteria. For the purposes of this study, patients should be re-evaluated for response every 8 weeks (every 2 cycles; 3 cycles for patients on study for a year). In addition to a baseline scan, confirmatory scans should also be obtained at least 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.

11.1.1 Definitions

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

Evaluable for objective response: All patients who received study drug will be considered evaluable for assessment of response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

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 one cycle of 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.

11.1.2 Disease Parameters

Measurable disease: Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥20 mm by chest x-ray or as ≥10 mm with CT scan, MRI, or calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Malignant lymph nodes: To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by CT scan (CT scan slice

thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable disease: All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Target lesions: All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions: All other lesions (or sites of disease) including any measurable lesions over and above the 5 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.

11.1.3 Guidelines 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.

Clinical lesions: Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and ≥10 mm diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Chest x-ray: Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

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 or less. If CT scans have slice thickness greater than 5 mm, 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).

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.

PET-CT: At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

Ultrasound: Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, Laparoscopy: The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

Tumor markers: Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published [*JNCI* 96:487-488, 2004; *J Clin Oncol* 17, 3461-3467, 1999; *J Clin Oncol* 26:1148-1159, 2008]. In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer [*JNCI* 92:1534-1535, 2000].

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 of any effusion 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.

FDG-PET: While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

a. Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
b. No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.
c. FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

Note: A 'positive' FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

11.1.4 Response Criteria

11.1.4.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum diameters.

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

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.

11.1.4.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis).

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

11.1.4.3 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.

**For Patients with Measurable Disease (i.e., Target Disease)**

| Target Lesions | Non-Target Lesions | New Lesions | Overall Response | Best Overall Response when Confirmation is Required* |
|---|---|---|---|---|
| CR | CR | No | CR | ≥4 wks. Confirmation** |
| CR | Non-CR/Non-PD | No | PR | ≥4 wks. Confirmation** |
| CR | Not evaluated | No | PR | |
| PR | Non-CR/Non-PD/not evaluated | No | PR | |
| SD | Non-CR/Non-PD/not evaluated | No | SD | Documented at least once ≥4 wks. from baseline** |
| PD | Any | Yes or No | PD | no prior SD, PR or CR |
| Any | PD*** | Yes or No | PD | |
| Any | Any | Yes | PD | |

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.
** Only for non-randomized trials with response as primary endpoint.
*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration.*" Every effort should be made to document the objective progression even after discontinuation of treatment.

**For Patients with Non-Measurable Disease (i.e., Non-Target Disease)**

| Non-Target Lesions | New Lesions | Overall Response |
|---|---|---|
| CR | No | CR |
| Non-CR/non-PD | No | Non-CR/non-PD* |
| Not all evaluated | No | not evaluated |
| Unequivocal PD | Yes or No | PD |
| Any | Yes | PD |

| |
|---|
| * 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised |

11.1.5 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.

## 12. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

### 12.1 Data Reporting

12.1.1 Method

This study will be monitored by the Clinical Trials Monitoring Service (CTMS). Data will be collected in the Center for Cancer Research C3D database and will be transmitted to CTMS electronically at least once every 2 weeks.

**Note**: **All** adverse events that have occurred on the study, including those reported through CTEP-AERS, must be reported via the monitoring method identified above.

12.1.2 Responsibility for Data Submission

N/A

### 12.2 CTEP Multicenter Guideline

N/A

### 12.3 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 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: E-mail: 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.

### 12.4 Genomic Data Sharing Plan

The NIH Genomic Data Sharing (GDS) Policy does not apply to this protocol as we will be performing exploratory genetic analysis of no more than 80 patient samples; therefore, this study does not meet GDS criteria, and a GDS plan is not warranted.

## 13. STATISTICAL CONSIDERATIONS

### 13.1 Study Design

The Phase I portion of the study will use a standard 3+ 3 design without intra-patient dose escalation. The MTD or recommended Phase II dose is the dose level at which no more than 1 of 6 patients experience DLT during the first cycle of treatment, and the dose below that at which at least 2 (of ≤ 6) patients have DLT as a result of the drug.

The Phase II portion will follow the Simon two-stage minimax design and will consist of three separately evaluated cohorts: patients with NSCLC, colon, and granulosa cell ovarian cancer. For NSCLC and colon, the maximum cohort size would be set at 25 evaluable patients. If at least 5 responses (at least 20%) are observed among the 25 evaluable patients, this regimen would be considered worthy of further testing in this disease. If no more than 1 response (no more than 6%) is observed among the initial 16 patients, the cohort would be terminated early and declared negative. This design yields at least 90% power to detect a true response rate of at

least 30%. It yields at least .90 probability of a negative result if the true response rate is no more than 10%, with at least .52 probability of early negative stopping. For granulosa cell ovarian cancer, maximum cohort size would be set at 15 evaluable patients. If at least 4 responses (at least 27%) are observed among the 15 evaluable patients, this regimen would be considered worthy of further testing in this disease. If no responses are observed among the initial 8 patients, the cohort would be terminated early and declared negative. This design yields at least 90% power to detect a true response rate of at least 40%. It yields at least .945 probability of a negative result if the true response rate is no more than 10%, with at least .43 probability of early negative stopping.

### 13.2 Sample Size/Accrual Rate

The Phase I portion of the study is designed to have 8 dose escalation cohorts, with a standard design using 3 patients per cohort, unless DLT is noted, in which point up to 6 patients may be enrolled in a cohort. In addition, up to an additional 15 patients will be enrolled at the MTD to further define the dose and evaluate PD studies at this dose level; with that number, and a tumor biopsy QA criteria failure rate of 50% with respect to paired (pre- and post-dose) biopsies, we have an 85% likelihood of having at least 6 usable PD samples, and 95% likelihood of having at least 5 usable samples. Biopsy tissue quality will be monitored and accrual will stop once we have obtained 6 usable paired samples. To allow for a small number of patients who may not be evaluable, the accrual ceiling for this trial is set at 65 (55 patients plus 10 healthy volunteers).

The Phase II portion of the study is designed to have up to 65 evaluable patients, and to allow for a small number of patients who may not be evaluable, the accrual ceiling for the Phase II portion is set at 75 patients. It is anticipated that 2-3 patients per month may be enrolled onto this study. Depending on whether the Phase I expansion and Phase II cohorts accrue to completion, it is expected that approximately 4 years will be required to accrue the number of patients necessary to complete the entire trial.

## 14. HUMAN SUBJECTS PROTECTIONS

### 14.1 Justification for Subject Selection

This study will be open to all individuals regardless of gender, ethnicity, or race, provided that the aforementioned inclusion and exclusion criteria are met. Patients for this study will be recruited through internal referral, our physician referral base, and through various cancer information hotlines (i.e., Clinical Studies Support Center, 1-800-4Cancer). To date, there is no information that suggests that differences in drug metabolism or effect on tumor would be expected in one ethnic group compared to another. Efforts will be made to extend accrual to each representative population, but a balance must be struck between participant safety considerations and limitations on the number of individuals exposed to potentially ineffective treatments on the one hand and the need to explore racial/ethnic aspects of clinical research on the other hand. If differences in outcome that correlate to ethnic identity are noted, a follow-up study may be written to investigate those differences more fully.

Due to lack of knowledge of the effects of TRC102 on the fetus or infants, as well as the possibility of teratogenic effects, pregnant and nursing women will be excluded from this trial. Patients with unstable or serious medical conditions are excluded due to the possibility that TRC102 may worsen their condition and the likelihood that the underlying condition may obscure the attribution of adverse events to TRC102. HIV-positive patients on combination antiretroviral therapy are excluded from the study because of possible PK interactions with TRC102.

14.1.1 Participation of Children

This study includes patients 18 years of age and older. Because insufficient dosing or adverse event data are currently available on the use of TRC102 in patients <18 years of age, children are excluded from this study, but may be eligible for future pediatric trials. Studies will be performed in patients <18 years of age when it is appropriate to do so.

## 14.2 Evaluation of Benefits and Risks/Discomforts

There may or may not be any clinical benefit to a patient from participation in this trial. Their participation will benefit future cancer patients. Potential risks include the possible occurrence of any of a range of side effects that are listed in the consent document. The procedure for protecting against or minimizing risks will be to medically evaluate patients as described in Sections 5 and 6. Although no compensation is available, any injury will be fully evaluated and treated in keeping with the benefits or care to which participants are entitled under applicable regulations.

## 14.3 Consent and Assent Process and Documentation

An associate or principal investigator on the trial will inform patients of the purpose, alternatives, drug administration plan, research objectives, and follow-up of this trial. The patient will be provided an IRB-approved consent for review and signature and his/her questions will be answered. After a decision is made to enroll into the study, a signature will be obtained from the patient. The original signed consent goes to Medical Records; a copy will be placed in the research record.

All patients must have a signed informed consent form and an on-study (confirmation of eligibility) form filled out and signed by a participating investigator before entering on study.

*Please note: As of **Amendment R (5/12/18)** healthy adult volunteers will no longer be recruited to provide blood for this study.* Healthy volunteers 18 years and older will give informed consent prior to undergoing blood collection. The study investigator will emphasize that there will be no benefit to them for entering into this study. Volunteers will be apprised of the risks of the study and be informed that the study is for research purposes only. Volunteers will be encouraged to ask questions and will sign the consent only if they choose to enter the study. The volunteer will sign the consent with a nurse or non-study participant as a witness while at NIH, prior to undergoing any study procedure.

14.3.1 Participation of subjects unable to give consent

Adults unable to give consent are excluded from enrolling in the protocol. However, re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (*i.e.*, long-term stabilization and/or improvement in the pain and physical impairment cause by cancer), all subjects ≥ age 18 at the NCI only will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team for evaluation. For those subjects that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in OHSRP Policy 403 for appointing a surrogate decision maker for adult subjects who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.

14.3.2 Informed Consent of non-English speaking subjects

If there is an unexpected enrollment of a research participant for whom there is no translated extant IRB approved consent document, the principal investigator and/or those authorized to obtain informed consent will use the Short Form Oral Consent Process as described in MAS Policy M77-2, OHSRP SOP 12, 45 CFR 46.117 (b) (2). The summary that will be used is the English version of the extant IRB approved consent document. Signed copies of both the English version of the consent and the translated short form will be given to the subject or their legally authorized representative, and the signed original will be filed in the medical record.

Unless the PI is fluent in the prospective subject's language, an interpreter will be present to facilitate the conversation (using the Short Form process). Preferably, someone who is independent of the subject (i.e., not a family member) will assist in presenting information and obtaining consent. Whenever possible, interpreters will be provided copies of the relevant consent documents well before the consent conversation with the subject (24 to 48 hours if possible).

14.3.3 As of **Amendment R** (5/12/2018), patients who have come off study but are still able to consent may be re-consented to the most recent protocol consent document if they wish to allow tumor tissue collected while on the trial to be used for the additional genomic analyses added in **Amendment R** (Section 9.3).

## 14.4 Procedure for Protecting Against or Minimizing Any Potential Risks

All care will be taken to minimize side effects, but they can be unpredictable in nature and severity. This study may involve risks to patients, which are currently unforeseeable. All patients will be monitored for side effects from taking study medication.

This study requires up to 3 CT-guided research tumor biopsies and up to 7 CT scans per year. These procedures confer radiation exposure at an effective dose of up to 10.1 rem per year. This dose is above NIH RSC guidelines of 5.0 rem per year in adults.

### 14.5 Patient Advocate

The patients' rights representative is available to patients receiving treatment on this protocol at the NIH Clinical Center at (301) 496-2626 in Building 10 of the Clinical Research Center, Room 1-3521, on the Bethesda NIH campus. Patients will be informed that they can contact the study PI or RN at any time with questions about their medical care, and that the patients' rights representative is also available to answer non-medical questions about the study.

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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: INFORMATION ON POSSIBLE DRUG INTERACTIONS

**Information on Possible Interactions with Other Agents for Patients and Their Caregivers and Non-Study Healthcare Team**

The patient _____ is enrolled on a clinical trial using the experimental agent TRC102 and temozolomide. This clinical trial is sponsored by the National Cancer Institute. This form is addressed to the patient, but includes important information for others who care for this patient.

TRC102 and temozolomide may interact with other drugs that are processed by your body. Because of this, it is very important to tell your study doctors about all of your medicine before you start this study. It is also very important to tell them if you stop taking any regular medicine, or if you start taking a new medicine while you take part in this study. When you talk about your medicine with your study doctor, include medicine you buy without a prescription at the drug store (over-the-counter remedy), or herbal supplements.

Many health care prescribers can write prescriptions. You must also tell your other prescribers (doctors, physicians' assistants, or nurse practitioners) that you are taking part in a clinical trial.

## APPENDIX C: PATIENT'S MEDICATION DIARY

INSTRUCTIONS

1. Complete one form for each cycle of treatment.
2. Swallow study drugs whole with a full glass of water either 1 hour before or 2 hours after a meal. Do not chew or open the capsules. If capsule is broken and the powder of the capsules gets on skin, wash the exposed area with as much water as necessary. Inform investigator or nurse if that occurs.
3. Record the date and time you took the drugs.
4. If you have any comments or notice any side effects, please record them in the Comments column.
5. Please bring this form and your bottle of drugs when you return for your appointment.
6. In case of errors, please place a single slash mark through the error and initial it. Please do not white out any error or scribble it out with ink. Please do not write the correct information directly over the error, but on a separate line next to the error.

# APPENDIX C: PATIENT'S MEDICATION DIARY

**Today's Date _____Cycle #_____ TRC102 Dose_____mg TMZ Dose_____mg**
**Patient Name_____(*initials acceptable)* Patient Study ID _____**

| Day | Date | Time of dose | | Number of Capsules Taken | | Comments |
|---|---|---|---|---|---|---|
| | | AM | PM | TRC102 | Temozolomide | |
| 1 | | | | | | |
| 2 | | | | | | |
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**Patient's signature: _____**

# APPENDIX D: PK/PD COLLECTION WORKSHEETS

**PHASE I RESEARCH URINE SAMPLE COLLECTION SHEET: CYCLE 1 DAY 1**

**CTEP Protocol**
**P9483** **Site:**
**Dose level:** **TRC102 Dose:**
**Patient ID:** **TMZ Dose:**

**Page ----------for sample pick-up**
**Lab phone: 301-451-1169**

**Research Nurse:**
Phone: Pager:
**PI: A. P. Chen, MD**
Phone: 301-768-2749

**PLEASE ENTER DATE, TIME, and VOLUME OF EACH VOID FOR 24 HOURS POST DRUG ADMINISTRATION**
**KEEP SAMPLES REFRIGERATED UNTIL PICKED UP**

| Date | Time | Volume of Void | | Record comments (i.e., if collection missed), and sign each time you collect a sample |
|---|---|---|---|---|
| **Pre-Drug Administration (SAMPLE #100)** | | | | |
| | | | | |
| **Administer TRC102 and Temozolomide, Time:** | | | | |
| **DAY 1 Start 24 hour urine collection immediately after study drug administration, keep on ice. Record volume of each void on this sheet and record total 24 hour volume at the end of the collection. At the end of 24 hours, retain 10 mL aliquot only, place on ice and discard remainder of urine. Charge urine collection container with acid as specified in Appendix E.** | | | | |
| **Date** | **Time (start of 24 hour collection time)** | **Volume of Void** | **24-hour urine collection** | **Record comments (i.e., if collection missed), and sign each time you collect a sample** |
| | | | **Volume 1** | |
| | | | **Volume 2** | |
| | | | **Volume 3** | |
| | | | **Volume 4** | |
| | | | **Volume 5** | |
| | | | **Volume 6** | |
| | | | **Volume 7** | |
| | | | **Volume 8** | |
| | | | **Volume 9** | |
| **Total (24-hour volume)** | | | **10 mL aliquot obtained ☐ (please check box)** | **Sample #101 (10 mL retained from total volume)** |
| **End 24-hour Urine Collection Time:** | | | | |

**Please Send a Copy of this sheet with the last specimen**

**Date:** **PHASE I PK/PD SAMPLE COLLECTION SHEET: Cycle 1 Day 1**

**Page ----------for Sample Pick-up**
**Lab phone: 301-451-1169**

**CTEP Protocol P9483** **Ht:** **Research Nurse:**

**Dose level:** **TRC102 Dose:** **Wt:** Phone: Pager:

**Patient ID:** **TMZ Dose:** **BSA:** **PI: A. P. Chen, MD**

Phone: 301-768-2749

*******ONLY PK SAMPLES GO ON ICE********

**PLEASE LABEL EACH TUBE WITH ACTUAL DATE AND TIME OF SAMPLE COLLECTION**

| Day | Time | Instructions | Ideal Time | Actual Time | Record comments (i.e., if collection missed), and sign each time you collect a sample |
|---|---|---|---|---|---|
| **Day 1** | **Prior to drug administration** | **PK 200** EDTA (lavender top) 2 mL<br>Label tube: drug, draw date and time<br>**PLACE ON ICE** | ----- | | |
| **Day 1** | **Prior to drug administration** | **PD 400** 4mL NaHep<br>Label tube: drug, draw date and time | | | |
| **Administer TMZ and TRC102, Time:** | | | | | |
| **Day 1** | **1 hour post dose** | **PK 201** EDTA (lavender top) 2 mL<br>Label tube: drug, draw date and time<br>**PLACE ON ICE** | | | |
| **Day 1** | **2 hours post dose** | **PK 202** EDTA (lavender top) 2 mL<br>Label tube: drug, draw date and time<br>**PLACE ON ICE** | | | |
| **Day 1** | **3 hours post dose** | **PK 203** EDTA (lavender top) 2 mL<br>Label tube: drug, draw date and time<br>**PLACE ON ICE** | | | |
| **Day 1** | **4 hours post dose** | **PK 204** EDTA (lavender top) 2 mL<br>Label tube: drug, draw date and time<br>**PLACE ON ICE** | | | |

*Continued over*

| Day | Time | Instructions | Ideal Time | Actual Time | Record comments (i.e., if collection missed), and sign each time you collect a sample |
|---|---|---|---|---|---|
| **Day 1** | **8 hours post dose** | **PK 205** EDTA (lavender top) 2 mL<br>Label tube: drug, draw date and time<br>**PLACE ON ICE** | | | |
| **Day 1** | **8 hours post dose** | **PD 401** 4 mL NaHep<br>Label tube: drug, draw date and time | | | |
| **Day 1** | **12 hours post dose** | **PK 206** EDTA (lavender top) 2 mL<br>Label tube: drug, draw date and time<br>**PLACE ON ICE** | | | |
| **Day 2** | **24 hours post dose** | **PK 207** EDTA (lavender top) 2 mL<br>Label tube: drug, draw date and time<br>**PLACE ON ICE** | | | |

**Date:** **PHASE I PK/PD SAMPLE COLLECTION SHEET: Cycle 1 Day 5**

**CTEP Protocol P9483** **Ht:**

**Dose level:** **TRC102 Dose:** **Wt:**

**Patient ID:** **TMZ Dose:** **BSA:**

**Page ----------**
**for Sample Pick-up**
**Lab phone: 301-451-1169**

**Research Nurse:**
Phone: Pager:

**PI: A. P. Chen, MD**
Phone: 301-768-2749

*******ONLY PK SAMPLES GO ON ICE********

**PLEASE LABEL EACH TUBE WITH ACTUAL DATE AND TIME OF SAMPLE COLLECTION**

| Day | Time | Instructions | Ideal Time | Actual Time | Record comments (i.e., if collection missed), and sign each time you collect a sample |
|---|---|---|---|---|---|
| **Day 5** | **Prior to dosing** | **PK 208** EDTA (lavender top) 2 mL<br>Label tube: drug, draw date and time<br>**PLACE ON ICE** | | | |
| **Day 5** | **Prior to dosing** | **PD 402** 4 mL NaHep<br>Label tube: drug, draw date and time | | | |

**Date:** **PHASE I PK/PD SAMPLE COLLECTION SHEET: Cycle 2 Day 1**

**CTEP Protocol P9483** **Ht:**
**Dose level:** **TRC102 Dose:** **Wt:**
**Patient ID:** **TMZ Dose:** **BSA:**
*******ONLY PK SAMPLES GO ON ICE********

**Page _____**
**for Sample Pick-up**
**Lab phone: 301-451-1169**

**Research Nurse:**
Phone: Pager:
**PI: A. P. Chen, MD**
Phone: 301-768-2749

**PLEASE LABEL EACH TUBE WITH ACTUAL DATE AND TIME OF SAMPLE COLLECTION**

| Day | Time | Instructions | Ideal Time | Actual Time | Record comments (i.e., if collection missed), and sign each time you collect a sample |
|---|---|---|---|---|---|
| **Day 1** | **Prior to dosing** | **PD 403** 4 mL NaHep<br>Label tube: drug, draw date and time | | | |

**PHASE I PK/PD SAMPLE COLLECTION SHEET: Day 1 each cycle/as determined by PI**

**Date:** **Cycle number: _____**

**CTEP Protocol P9483** **Ht:**
**Dose level:** **TRC102 Dose:** **Wt:**
**Patient ID:** **TMZ Dose:** **BSA:**
*******ONLY PK SAMPLES GO ON ICE********

**Page**
**for Sample Pick-up**
**Lab phone: 301-451-1169**

**Research Nurse:**
Phone: Pager:
**PI: A. P. Chen, MD**
Phone: 301-768-2749

**PLEASE LABEL EACH TUBE WITH ACTUAL DATE AND TIME OF SAMPLE COLLECTION**

| Day | Time | Instructions | Ideal Time | Actual Time | Record comments (i.e., if collection missed), and sign each time you collect a sample |
|---|---|---|---|---|---|
| **Day 1** | **Prior to dosing** | **PD 40X** 4 mL NaHep<br>Label tube: drug, draw date and time | | | |

**Date:** **PHASE II PD SAMPLE COLLECTION SHEET: Cycle 1 Day 1**

**CTEP Protocol P9483** **Ht:**
**Dose level:** **TRC102 Dose:** **Wt:**
**Patient ID:** **TMZ Dose:** **BSA:**

**Page ----------**
**for Sample Pick-up**
**Lab phone: 301-451-1169**

**Research Nurse:**
Phone: Pager:
**PI: A. P. Chen, MD**
Phone: 301-768-2749

**PLEASE LABEL EACH TUBE WITH ACTUAL DATE AND TIME OF SAMPLE COLLECTION**

| Day | Time | Instructions | Ideal Time | Actual Time | Record comments (i.e., if collection missed), and sign each time you collect a sample |
|---|---|---|---|---|---|
| **Day 1** | **Prior to drug administration** | **400** 10 mL Streck<br>Label tube: drug, draw date and time | | | |

**Date:** **PHASE II PK/PD SAMPLE COLLECTION SHEET: Cycle 1 Day 4 or 5 (day of biopsy)**

**CTEP Protocol P9483** **Ht:**
**Dose level:** **TRC102 Dose:** **Wt:**
**Patient ID:** **TMZ Dose:** **BSA:**

**Page _____**
**for Sample Pick-up**
**Lab phone: 301-451-1169**

**Research Nurse:**
Phone: Pager:
**PI: A. P. Chen, MD**
Phone: 301-768-2749

**PLEASE LABEL EACH TUBE WITH ACTUAL DATE AND TIME OF SAMPLE COLLECTION**

| Day | Time | Instructions | Ideal Time | Actual Time | Record comments (i.e., if collection missed), and sign each time you collect a sample |
|---|---|---|---|---|---|
| **Day 4/5** | **Prior to dosing** | **401** 10 mL Streck<br>Label tube: drug, draw date and time | | | |

**Date:** **PHASE II PK/PD SAMPLE COLLECTION SHEET: Day 1 (±1 day) each**

**cycle/as determined by PI**

**Cycle number: _____**

| | | | | |
|---|---|---|---|---|
| **CTEP Protocol P9483** | | **Ht:** | **Page for Sample Pick-up Lab phone: 301-451-1169** | **Research Nurse:** Phone: Pager: **PI: A. P. Chen, MD** Phone: 301-768-2749 |
| **Dose level:** | **TRC102 Dose:** | **Wt:** | | |
| **Patient ID:** | **TMZ Dose:** | **BSA:** | | |

**PLEASE LABEL EACH TUBE WITH ACTUAL DATE AND TIME OF SAMPLE COLLECTION**

| Day | Time | Instructions | Ideal Time | Actual Time | Record comments (i.e., if collection missed), and sign each time you collect a sample |
|---|---|---|---|---|---|
| **Day 1** | **Prior to dosing** | **40__** 10 mL Streck<br>Label tube: drug, draw date and time | | | |

**Date:** **PHASE II PK/PD SAMPLE COLLECTION SHEET: Day of restaging follow-up or progression biopsy**

| | | | | |
|---|---|---|---|---|
| **CTEP Protocol P9483** | | **Ht:** | **Page _____ for Sample Pick-up Lab phone: 301-451-1169** | **Research Nurse:** Phone: Pager: **PI: A. P. Chen, MD** Phone: 301-768-2749 |
| **Dose level:** | **TRC102 Dose:** | **Wt:** | | |
| **Patient ID:** | **TMZ Dose:** | **BSA:** | | |

**PLEASE LABEL EACH TUBE WITH ACTUAL DATE AND TIME OF SAMPLE COLLECTION**

| Day | Time | Instructions | Ideal Time | Actual Time | Record comments (i.e., if collection missed), and sign each time you collect a sample |
|---|---|---|---|---|---|
| **Day X** | **Prior to dosing** | **40__** 10 mL Streck<br>Label tube: drug, draw date and time | | | |

**Date:** **PHASE II PK/PD SAMPLE COLLECTION SHEET: Optional blood for germline WES, collected any time while on trial**

| | | | | |
|---|---|---|---|---|
| **CTEP Protocol P9483** | | **Ht:** | **Page _____ for Sample Pick-up Lab phone: 301-451-1169** | **Research Nurse:** Phone: Pager: **PI: A. P. Chen, MD** |
| **Dose level:** | **TRC102 Dose:** | **Wt:** | | |
| **Patient ID:** | **TMZ Dose:** | **BSA:** | | |

Phone: 301-768-2749

**PLEASE LABEL EACH TUBE WITH ACTUAL DATE AND TIME OF SAMPLE COLLECTION**

| Day | Time | Instructions | Actual Day | Actual Time | Record comments (i.e., if collection missed), and sign each time you collect a sample |
|---|---|---|---|---|---|
| **Any day** | **Any time** | **001** 10 mL Streck<br>Label tube: drug, draw date and time | | | |

## APPENDIX E: PK SAMPLE HANDLING

### PLASMA

The 2-mL blood sample is immediately placed on ice and centrifuged within 30 minutes of collection to separate the plasma. The plasma is divided between two tubes, one of which is labeled "A" (which is frozen and stored at -70°C until analyzed) and the other is labeled "B," which contains 25 µL of commercially available 1N hydrochloric acid (HCl): sample "B" is then mixed by vortex action, frozen, and stored at -70°C until analysis.

### URINE

A pre-dose void is collected, its volume measured and recorded, and then a 10-mL aliquot of urine is removed to which 0.4 mL of 1N hydrochloric acid is added, mixed, frozen and stored at -70°C until analysis.

The plastic container used for the collection/storage of all voids over the 24-hr collection period is first charged with 25 mL of commercially available 1N HCl prior to the introduction of any patient urine. An additional 25 mL of 1N HCl is added for each 500 mL of urine collected. The contents of the container are mixed by swirling.

Each void volume is first measured before adding to the container.

At the conclusion of collecting all voids, the contents of the container are mixed by swirling, and then a 10-mL aliquot is removed, frozen, and stored at -70°C until analysis.

Send plasma and urine samples to:

Tracy W. Webb
Office of the Associate Director/DTP/NCI
FNLCR
Boyles Street
Building 1047, Room 8
Frederick, MD 21702
301-846-7402
webbtw@mail.nih.gov

## APPENDIX F: ERYTHROCYTE ANALYSIS

*Please note: As of **Amendment R (5/12/18)**, we will no longer perform this analysis of the mechanisms of extravascular hemolysis.*

Blood will be collected for the analysis below in collaboration with the hematology attending consult team. These studies are optional. Blood volumes may be changed at the PI/consult team's discretion. Additional studies (Folate, Vitamin B12, Erythropoietin, TSH) may be done as clinically indicated based on baseline Hgb values.

Blood collection tubes, volumes, and service for each test can be found at the Dept. of Transfusion Medicine's Test Guide: http://cclnprod.cc.nih.gov/dlm/testguide.nsf/

When patients develop Hgb level decrement of more than 2 g/dL of baseline during cycle follow-up, contact the hematology consult team for official consultation (Dr. Kazusa Ishii, kazusa.ishii@nih.gov; 301-496-5093); the hematology attending consult team will develop a reference guide/SOP. The hematology consult team will order necessary labs, review the peripheral blood smear, and obtain systematic relevant hematological history at the time of consultation.

Laboratory testing/ timing

**1) Baseline (**within 72 hours prior to enrollment)

- CBC with WBC differential
- Reticulocyte count
- Peripheral blood smear evaluation
- LDH
- LFTs
- Haptoglobin
- Ferritin, iron study
- DAT (Direct Coombs test)
- Renal function
- Urinalysis with microscopy, urine urobilinogen
- G6PD
- Spleen size evaluation (if CT is done recently per protocol, there is no need to repeat. If not, then US spleen)
- Osmotic fragility test
- RBC enzyme evaluation
- Methemoglobin, venous

**2) C1D7 +/- 2 days**

- CBC with diff
- Reticulocyte count
- Peripheral blood smear
- LDH

- LFTs
- Haptoglobin
- DAT
- Ferritin, iron study
- Urinalysis with microscopy, urine urobilinogen
- RBC enzyme evaluation
- Osmotic fragility test
- Methemoglobin, venous

**3) C1D14 +/- 2 days, C1D21 +/- 2 days**

- CBC with diff
- Reticulocyte count
- Peripheral blood smear
- LDH
- LFTs
- Haptoglobin
- DAT
- Urinalysis with microscopy, urine urobilinogen