To: CTEP Protocol and Information Office

From: Matthew Ingham, M.D.

Date: February 14, 2023

Re: Amendment #8 for Protocol 10250

SUMMARY OF CHANGES – Protocol

I: Protocol Changes by Principal Investigator:

#	Section	Comments
1.	Title Page	The overall PI has been changed to Dr. Brian Van Tine of Washington University. The lead LAO remains the same.
2.	Calendar	For patients who remain on the study as of February 2023 (and have been on treatment more than 2 years), the study calendar was modified to require in-person visits every other cycle, and permit telephone or TeleHealth during the intervening visits. Laboratory tests must still be performed. This modification was made at the request of the site/treating investigators.

NCI Protocol #: 10250

Local Protocol #: Columbia University Medical Center IRB #AAAS4040

ClinicalTrials.gov Identifier: NCT03880019

TITLE: A Phase II Study of the PARP Inhibitor Olaparib in Combination with the DNA Damaging Agent Temozolomide for the Treatment of Advanced Uterine Leiomyosarcoma

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NCI-Supplied Agent(s): Olaparib (NSC 747856)

Other Agent(s): Temozolomide (NSC 362856), Commercial

IND #: [redacted]
IND Sponsor: DCTD, NCI

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Original / August 1, 2018
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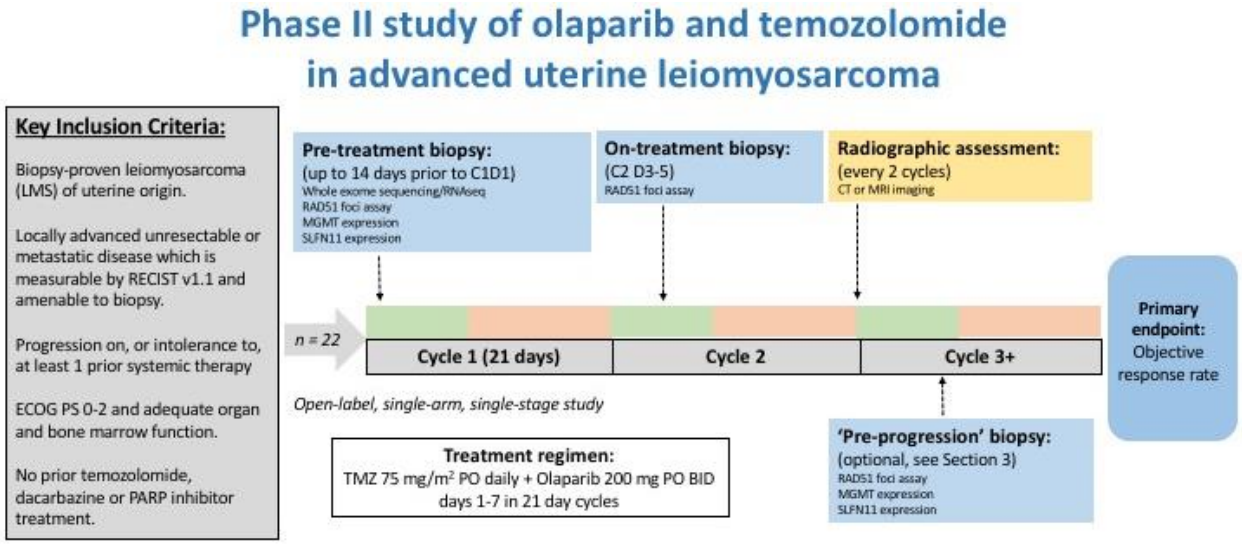
Amendment 3 / April 16, 2020
Amendment 4 / April 23, 2021
Amendment 5 / September 14, 2021
Amendment 6 / February 18, 2022
Amendment 7 / June 27, 2022
Amendment 8 / February 14, 2023

SCHEMA



LMS=Leiomyosarcoma, TMZ=temozolomide, BID=twice a day, MGMT=O[6]-methylguanine-DNA methyltransferase, SLFN11=Schlafen family member number 11, RAD51= DNA repair protein RAD51 homolog, ECOG=Eastern Cooperative Oncology Group, PS=performance status.

NOTE: For patients who continue beyond 12 cycles, Radiographic assessment (MRI or CT imaging) may be changed to once every 4 cycles (12 weeks) ± 3 days versus once every 2 cycles.

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1. OBJECTIVES

1.1 Primary Objective

1.1.1 To evaluate whether combination treatment with temozolomide (TMZ) + olaparib shows preliminary evidence of clinical activity among patients with advanced uterine leiomyosarcoma (LMS) as measured by the confirmed objective response rate (ORR). .

1.2 Secondary Objectives

1.2.1 To evaluate the toxicity profile associated with the combination treatment.

1.2.2 To evaluate the progression free survival (PFS) associated with the combination treatment.

1.2.3 To evaluate what proportion of uterine LMS tumors exhibit homologous recombination (HR) deficiency as measured by (1) genomic alterations in HR components at baseline and (2) DNA repair protein RAD51 homolog (RAD51) foci formation at baseline and while on study treatment. To evaluate the feasibility of these assays in human tissue, and to preliminarily evaluate for any association between presence of HR deficiency as measured by each assay and increased clinical benefit from the study treatment.

1.3 Exploratory Objectives

1.3.1 To evaluate what proportion of uterine LMS tumors exhibit HR deficiency as measured by Schlafen family member number 11(SLFN11) protein expression at baseline. To evaluate the feasibility of this assay in human tissue, and to preliminarily evaluate for any association between presence of HR deficiency as measured by this assay and increased clinical benefit from the study treatment.

1.3.2 To evaluate MGMT protein expression in uterine LMS tumors, and to preliminarily evaluate for any association between MGMT expression and increased clinical benefit from the study treatment.

1.3.3 To perform an optional third tissue biopsy in patients who initially benefit from study treatment but later show early evidence of disease progression to evaluate for changes in the status of the RAD51 foci, MGMT, and SLFN11 assays at that time.

2. BACKGROUND

2.1 Study Disease

Soft tissue sarcoma (STS) is a heterogeneous malignancy of mesenchymal origin comprised of more than 50 subtypes with distinct biologic features. LMS is a clinically aggressive sarcoma subtype arising from smooth muscle and most often presents in the uterus or retroperitoneum.

LMS, a relatively common sarcoma subtype, accounts for up to 20% of new STS diagnoses (George *et al.*, 2018). Although a subset of cases are surgically resectable at presentation, local recurrence or metastatic relapse occurs in approximately 40% of patients (Coindre *et al.*, 2001). Advanced LMS is treated with chemotherapy. The most active regimens, doxorubicin or the combination of gemcitabine with docetaxel, are associated with ORR of 15-35% and median PFS of 4-6 months (Hensley *et al.,* 2008; Sutton *et al.,* 2005). Recently, the combination of doxorubicin with the novel platelet derived growth factor receptor alpha (PDGFRα) monoclonal antibody, olaratumab, was shown to prolong survival as compared doxorubicin monotherapy in a phase 2 study of unselected soft tissue sarcoma subtypes and received FDA approval (Tap *et al.,* 2016). However, response rates for this regimen remain under 20% and, as multiple subtypes were included on this study, the specific benefit for LMS remains uncertain. The cytotoxic agent trabectedin and the angiogenic receptor tyrosine kinase inhibitor pazopanib are the other therapies approved for LMS and provide a PFS benefit of several months with no improvement in overall survival (OS) (van der Graaf *et al.,* 2012; Demetri *et al.,* 2016). Dacarbazine, and the related oral agent temozolomide, are other cytotoxic agents used in the advanced setting. Unfortunately, LMS appears resistant to immune checkpoint blockade with anti-PD-1 monotherapy, with an ORR of 0% in a recent phase 2 study with pembrolizumab (Burgess *et al.,* 2017). Thus, LMS represents a relatively common sarcoma subtype in particular need of new treatments which reflect the cancer biology of this specific sarcoma.

We propose the combination of olaparib, a potent polyadenosine 5'diphosphoribose (poly [ADP ribose]) polymerization (PARP) inhibitor (PARPi), with TMZ, a DNA alkylating agent, as a novel treatment for uterine LMS based on compelling preclinical data. This proposal is further strengthened noting that TMZ is a standard of care agent with monotherapy activity in LMS and that emerging evidence suggests LMS is a sarcoma subtype with a "BRCAness" phenotype and is deficient in DNA damage repair (and, specifically, homologous recombination).

2.2 CTEP IND Agent

2.2.1 Olaparib (AZD2281)

Olaparib (AZD2281, KU-0059436) is a potent PARPi (PARP-1, -2 and -3) that is being developed as an oral therapy, both as a monotherapy (including maintenance) and for combination with chemotherapy and other anti-cancer agents.

PARP inhibition is an approach targeting tumors with deficiencies in DNA repair mechanisms. PARP enzymes are essential for repairing DNA single strand breaks (SSBs). Inhibiting PARPs leads to the persistence of SSBs, which are then converted to the more serious DNA double strand breaks (DSBs) during the process of DNA replication. During the process of cell division, DSBs can be efficiently repaired in normal cells by homologous recombination repair (HR). Tumors with HR deficiencies (HRD), such as ovarian cancers in patients with BRCA1/2 mutations, cannot accurately repair the DNA damage, which may become lethal to cells as it accumulates. In such tumor types, olaparib may offer a potentially efficacious and less toxic cancer treatment compared with currently available chemotherapy regimens.

BRCA1 and BRCA2 defective tumors are intrinsically sensitive to PARP inhibitors, both in tumor models *in vivo* (Rottenberg *et al*., 2008; Hay *et al*., 2009) and in the clinic (Fong *et al*., 2009). The mechanism of action for olaparib results from the trapping of inactive PARP onto the single-strand breaks (SSBs) preventing their repair (Helleday, 2011; Murai *et al*., 2012). Persistence of SSBs during DNA replication results in their conversion into the more serious DNA DSBs that would normally be repaired by HR repair. Olaparib has been shown to inhibit selected tumor cell lines *in vitro* and in xenograft and primary explant models as well as in genetic BRCA knock-out models, either as a stand-alone treatment or in combination with established chemotherapies.

2.2.1.1 Mechanism of Action

The mechanism of action for olaparib activity as a single agent has been proposed to involve the trapping of inactivated PARP onto the single-strand breaks preventing their repair and generating a potential block for cellular DNA replication (Helleday, 2011; Murai *et al.,* 2012). An important consequence of this is that processing of trapped PARP-DNA complexes and/or the stalling of replication forks, or collapsing of replication forks, is predicted to lead to the generation of the more serious DNA DSBs. These DSBs would normally be repaired by a process that involves ATM (a major DNA DSB signaling kinase, the 'MRN' nuclease protein complex; made up of Meiotic Recombination 11 Homolog A [MRE11A], human RAD50 homolog [RAD50] and Nijmegen breakage syndrome1 [NBS1]) and additional homologous recombination DNA repair (HRR) proteins such as DNA repair protein RAD51 homolog (RAD51), BRCA1 and BRCA2.

In some instances where DNA repair defects may not result in the same level of sensitivity as *BRCA* mutations, and therefore single agent olaparib treatment may not be sufficient to induce cell kill through synthetic lethality, it may still be possible to induce tumor cell death through combinations with ionizing radiation or chemotherapies that either increase DNA damage accumulation or mitotic stress. The latter is relevant since it is often during mitosis that unrepaired DNA damage leads to cell death through a process known as mitotic catastrophe (Castedo *et al.,* 2004).

2.2.1.2 Nonclinical Summary

Olaparib is a potent inhibitor of mammalian PARP-1, PARP-2 and PARP-3 and inhibits selected tumor cell lines *in vitro* and xenograft tumor growth *in vivo*, either as a stand-alone treatment or in combination with established chemotherapies (Investigator's Brochure, 2018). Olaparib has a half-maximal inhibitory concentration (IC_{50}) against the PARP-1 enzyme of 5 nM, an IC_{50} against the PARP-2 enzyme of 1 nM, and an IC_{50} against the PARP-3 enzyme of 4 nM. The main target of olaparib that is responsible for the induction of synthetic lethality in HRD cancer cells is PARP-1. In cells cultured *in vitro*, 50% inhibition of PARP-1 activity, as measured by the formation of PAR, occurs at concentrations of 6-8 nM, with greater than 90% PAR formation being inhibited between 30-100 nM and complete inhibition of PARP-1 activity occurring between 100-300 nM. *In vivo*, the PARP-1 IC_{50} was 120 nM (standard error ±33 nM) and the IC_{90} was 576 nM (standard error not calculated). Olaparib doses leading to tumor regression in a BRCA2 mutant PDX model resulted in PARP-1 inhibition of >50% for more than 10 hours and

inhibition of >90% for more than 4 hours. Analysis of a panel of cancer cell lines identified those deficient in homologous recombination repair factors, notably BRCA1 and BRCA2, as being particularly sensitive to treatment with olaparib.

In vitro, olaparib showed no significant off-target activity at concentrations 6-times the human mean free C_{max} at the clinical dose of 300 mg twice a day (BID) (3.65 mcM) when screened against a diverse panel of molecular targets including enzymes, receptors, transporters and ion channels (Investigator's Brochure, 2018).

In vivo, olaparib was rapidly absorbed and the absolute bioavailability of olaparib following oral (PO) dosing was 60% in mice, 20% in rats, and 80% in dogs (Investigator's Brochure, 2018). The major isozymes responsible for the metabolism of olaparib are CYP3A4 and CYP3A5, with the majority of the metabolism resulting in oxidation and hydroxylation processes with the main site of metabolism being the piperazine and fluorobenzyl ring structures. *In vitro*, olaparib was found to be both a direct inhibitor (IC_{50} 119 mcM) and a time dependent inhibitor (inactivation rate constant [K_{inact}] 0.0675 min^{-1}; inhibition constant [K_i] 72.2 mcM) of CYP3A. Excretion of the drug was predominately in the feces in both the rat and the dog.

Repeat dose and reproductive toxicology studies were conducted in both rats and dogs with exposure levels below those achieved at the clinical therapeutic 300 mg BID (tablet) dose (Investigator's Brochure, 2018). The repeat-dose toxicology studies of up to 6 months found that the primary target organ for toxicity was the bone marrow, with associated changes in peripheral hematology parameters, which may be related to the primary pharmacology of olaparib. All changes showed full or partial recovery following withdrawal of olaparib. Administration of olaparib during organogenesis caused reductions in early embryofetal survival and fetal weights as well as increases in the incidence of visceral and skeletal abnormalities and major eye and vertebral/rib malformations at dose levels that were not maternally toxic.

Combination of olaparib with the mono-methylating agent TMZ or topotecan suggest the potentiation of anti-tumor activity in a number of cell lines and in rats with no additional target organ toxicities compared to single agent administration (Investigator's Brochure, 2018).

2.2.1.3 Summary of Clinical Experience

As of December 15, 2017, approximately 8319 patients have received olaparib in Phase 1, 2, and 3 trials (Investigator's Brochure, 2018). Of the 4575 patients on AstraZeneca-sponsored trials, 1512 patients received the capsule formulation and 3038 received the tablet formulation and 25 patients received both the capsule and tablet. In company sponsored trials, 3133 patients have received olaparib as a monotherapy and 1442 patients received olaparib in combination with chemotherapy or other anti-cancer agents including patients that received the combination therapy sequentially to receiving the monotherapy. The Phase 3 registration studies and most new clinical studies are investigating the tablet formulation which delivers the therapeutic dose of olaparib in fewer dose units than the capsule. The tablet formulation was registered for use in the US in August 2017 for ovarian cancer and in January 2018 for breast cancer. In January 2018, the tablet formulation was also registered for use in Japan for ovarian cancer. The recommended olaparib monotherapy tablet dose under investigation is 300 mg BID.

2.2.1.4 Clinical Pharmacokinetics and Metabolism

As of December 15, 2017, there is pharmacokinetic (PK) data from 11 clinical studies. Olaparib is orally available and the tablet formulation is rapidly absorbed, reaching peak plasma concentration (t_{max}) in 1.5 hours (Investigator's Brochure, 2018). The plasma concentrations decline in a biphasic manner with an average terminal elimination half-life ($t_{1/2}$) of 14.9 hours (standard deviation [S_d] 8.2 hours). The mean apparent oral clearance rate was approximately 7.4 L/hour (S_d 3.9 L/hour). The mean volume of distribution (V_d) of olaparib following a single administration of 300 mg oral tablet dose was 158 L (S_d 136 L) indicating olaparib is distributed into the tissues.

The metabolism of olaparib was assessed in a study of six female patients ranging in age from 34-72 years (Investigator's Brochure, 2018). Upon the administration of a single 100 mg dose of olaparib containing 1x50 mg capsule of radiolabeled [^{14}C]-olaparib and 1x50 mg capsule of unlabeled olaparib, 70% of the radioactivity in the blood was olaparib. Three metabolites each comprised around 10% of the material: M12 (9.3%, ring-open piperazin-3-ol), M15 (10.3%, 4-fluorophenol (hydroxy)methyl), and M18 (13.7%, piperazin 3-ol).

Olaparib is eliminated in both the urine (44% of the dose) and feces (42% of the dose) (Investigator's Brochure, 2018). In the urine, olaparib was the most abundant component, accounting for between 10% and 19% of the dosed material. Up to 37 further drug-related components were observed, 18 of which were quantifiable by high performance liquid chromatography with mass spectrometric detection (HPLC/MS) with radioactivity detection. At least 20 components were observed in the pooled fecal samples with 6 components accounting for >1% of the dose and the remaining metabolites detectable only by HPLC/MS. The major components present were unchanged olaparib (0.6 to 14% of the dose) and M15 (0.9 to 8% of the dose). Four further metabolites (M9, M12, M23 and M25) were also prominent each accounting for ≤6% of the dose.

The PK parameters for multiple dosing of olaparib are well predicted from the single dose studies. There is not extensive accumulation with multiple dosing. At 300 mg oral dose BID, the temporal change parameter (AUC at steady state/AUC following a single dose) was approximately 1.5 (S_d 0.6) (Investigator's Brochure, 2018).

At capsule doses ≥40 mg, the inhibition of PARP-1 measured in peripheral blood mononuclear cells (PBMC) reached its maximum of 50% to 60% of the baseline, and this level of inhibition was achieved by 6 hours after the first dose of olaparib and was maintained with repeated dosing (Investigator's Brochure, 2018). In tumor biopsies from 60 breast cancer patients who received doses of olaparib between 10 mg BID and 400 mg BID, PARP inhibition ranged from 20% to 80% and did not relate to the dose.

2.2.1.5 Clinical Safety Summary

Olaparib monotherapy has been associated with adverse reactions generally of mild or moderate severity (Common Terminology Criteria for Adverse Events v.5.0 [CTCAE] grade 1 or 2) and

generally not requiring treatment discontinuation (Investigator's Brochure, 2018). In a pool of 1248 patients, the most frequently observed adverse reactions across clinical trials in patients receiving olaparib monotherapy (≥10%) were nausea, vomiting, diarrhea, dyspepsia, fatigue, headache, dysgeusia, decreased appetite, dizziness, and anemia. Anemia and other hematologic toxicities were generally low grade (grade 1 or 2). However, anemia was the most commonly reported grade ≥3 adverse event reported in clinical trials. The median time to first report of anemia was approximately 4 weeks (approximately 7 weeks for grade ≥3). Anemia can be managed with dose interruptions, dose reductions, and blood transfusions where appropriate. In one phase 3 trial in ovarian cancer patients, the incidence of anemia was 43.6% of patients, with grade ≥3 in 19.5% of patients, leading to dose interruptions (16.9%), dose reductions (8.2%), discontinuation of treatment (3.1%). In addition, 17.9% of patients treated with olaparib required one or more blood transfusions during treatment.

In clinical studies with olaparib the incidence of grade ≥2 shifts (elevations) from baseline in blood creatinine was approximately 15% (Investigator's Brochure, 2018). Data from a double-blind placebo-controlled study showed median increase up to 23% from baseline remaining consistent over time and returning to baseline after treatment discontinuation, with no apparent clinical sequelae; 90% of patients had creatinine values of grade 0 at baseline and 10% were grade 1 at baseline.

Nausea was generally reported very early, with first onset within the first month of olaparib treatment in the majority of patients (Investigator's Brochure, 2018). Vomiting was reported early, with first onset within the first 2 months of olaparib treatment in the majority of patients. Both nausea and vomiting were reported to be intermittent for the majority of patients and can be managed by dose interruption, dose reduction and/or antiemetic therapy. Antiemetic prophylaxis is not required.

Studies of olaparib in combination with various chemotherapy agents indicate an increase in bone marrow toxicity (anemia, neutropenia, thrombocytopenia) greater than expected if the agents had been administered alone (Investigator's Brochure, 2018). The effects are generally transient, but treatment delays are common and alternative administration schedules/toxicity management processes have been evaluated within some of these studies. When this type of toxicity has occurred, it has been managed by routine clinical practice including dose delays, dose reductions, intermittent dosing and/or the use of supportive care measures, including granulocyte colony stimulating factor (G-CSF).

The incidence of myelodysplastic syndrome/acute myeloid leukemia (MDS/AML) in patients treated in clinical trials with olaparib monotherapy, including long-term survival follow-up, was <1.5% and the majority of events had a fatal outcome (Investigator's Brochure, 2018). If MDS and/or AML are confirmed while on treatment with olaparib, it is recommended that olaparib should be discontinued and the patient be treated appropriately.

Pneumonitis has been reported in <1.0% patients treated with olaparib monotherapy in clinical studies (Investigator's Brochure, 2018). When olaparib was used in clinical studies in combination with other therapies there have been events with a fatal outcome. If patients present with new or worsening respiratory symptoms such as dyspnea, cough and fever, or an abnormal

chest radiologic finding is observed, olaparib treatment should be interrupted and prompt investigation initiated. If pneumonitis is confirmed, olaparib treatment should be discontinued and the patient treated appropriately.

2.2.1.6 Clinical Efficacy Summary

There have been many clinical trials looking at the efficacy of olaparib both as a monotherapy as well as in combination with different chemotherapy treatments (Investigator's Brochure, 2018). As a monotherapy, olaparib has shown statistically significant increases in progression-free survival (PFS) in both Phase 2 and Phase 3 trials in advanced ovarian cancer. In a Phase 3 study comparing olaparib to chemotherapy of the physician's choice in BRCA mutated, HER-2 negative breast cancer, olaparib increased the PFS and objective response rate.

Olaparib in combination with paclitaxel has increased the overall survival (OS) of patients in Phase 2 and a confirmatory Phase 3 trial in 2nd line gastric cancer. In both trials, there was no difference in the PFS (Investigator's Brochure, 2018).

Olaparib in combination with trabectedin showed partial response or stable disease in 9 out of 22 patients with STS including LMS and Ewing Sarcoma (ES) in a phase 1b study (Grignani *et al*. 2016).

In non-comparative studies, olaparib has shown anti-tumor activity in patients with germline *BRCA* mutated cancers including ovarian, breast, pancreatic, and prostate (Investigator's Brochure, 2018). In a phase 2 study of ES, no significant responses or durable disease control was observed (Choy *et al*. 2014).

The recommended monotherapy dose of olaparib is 300 mg BID (Investigator's Brochure, 2018).

2.3 Commercial Agent

2.3.1 Temozolomide

2.3.1.1 Mechanism of Action

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 (Package Insert, 2017).

2.3.1.2 Clinical Pharmacokinetics and Metabolism

2.3.1.2.1 Absorption and Distribution

TMZ is rapidly and completely absorbed after oral administration with a peak plasma concentration (C_{max}) achieved in a median T_{max} of 1 hour (Package Insert, 2017). Food reduces the rate and extent of TMZ absorption. Mean peak plasma concentration and AUC decreased by

32% and 9%, respectively, and median T_{max} increased by 2-fold (from 1-2.25 hours) when TMZ was administered after a modified high-fat breakfast. A PK study comparing oral and intravenous (IV) TMZ in 19 patients with primary CNS malignancies showed that 150 mg/m^2 TMZ for injection administered over 90 minutes is bioequivalent to 150 mg/m^2 TMZ oral capsules with respect to both C_{max} and AUC of TMZ and MTIC. Following a single 90-minute IV infusion of 150 mg/m^2, the geometric mean C_{max} values for TMZ and MTIC were 7.3 mcg/mL and 276 ng/mL, respectively. Following a single oral dose of 150 mg/m^2, the geometric mean C_{max} values for TMZ and MTIC were 7.5 mcg/mL and 282 ng/mL, respectively. Following a single 90-minute IV infusion of 150 mg/m^2, the geometric mean AUC values for TMZ and MTIC were 24.6 mcg·hr/mL and 891 ng·hr/mL, respectively. Following a single oral dose of 150 mg/m^2, the geometric mean AUC values for TMZ and MTIC were 23.4 mcg·hr/mL and 864 ng·hr/mL, respectively. TMZ has a mean apparent V_d of 0.4 L/kg (%CV=13%). It is weakly bound to human plasma proteins; the mean percent bound of drug-related total radioactivity is 15%.

2.3.1.2.2 Metabolism and Elimination

TMZ is spontaneously hydrolyzed at physiologic pH to the active species, MTIC and to TMZ acid metabolite (Package Insert, 2017). MTIC is further hydrolyzed to 5-amino-imidazole-4-carboxamide (AIC), which is known to be an intermediate in purine and nucleic acid biosynthesis, and to methylhydrazine, which is believed to be the active alkylating species. Cytochrome P450 enzymes play only a minor role in the metabolism of TMZ and MTIC. Relative to the AUC of TMZ, the exposure to MTIC and AIC is 2.4% and 23%, respectively.

2.3.1.2.3 Excretion

About 38% of the administered TMZ total radioactive dose is recovered over 7 days: 37.7% in urine and 0.8% in feces (Package Insert, 2017). The majority of the recovery of radioactivity in urine is unchanged temozolomide (5.6%), AIC (12%), TMZ acid metabolite (2.3%), and unidentified polar metabolite(s) (17%). Overall clearance of TMZ is about 5.5 L/hr/m^2. TMZ is rapidly eliminated, with a mean elimination half-life of 1.8 hours, and exhibits linear kinetics over the therapeutic dosing range of 75 to 250 mg/m2 /day.

2.4 Rationale for Combination Treatment

We chose to further evaluate olaparib + TMZ in sarcoma and found this combination particularly efficacious in uterine LMS models. The clinical development of this combination in uterine LMS is further supported by the following observations:

(A) TMZ is a standard of care agent with monotherapy efficacy in LMS: Dacarbazine, an IV chemotherapy with analogous mechanism of action to TMZ, is FDA-approved for advanced STS, and TMZ is listed on the National Comprehensive Cancer Network (NCCN) guidelines as a standard of care treatment for STS. In clinical studies, dacarbazine's efficacy appeared most pronounced in LMS as compared other subtypes (Buesa *et al.,* 1991; Gottlieb *et al.,* 1976). In clinical practice, TMZ, owing to an oral route of administration, is frequently substituted for dacarbazine as a later-line treatment for LMS. In a phase 2 study of TMZ in STS, 45 patients

received TMZ at doses of 75–100 mg/m^2/day and TMZ had a modest ORR and PFS as monotherapy in LMS (Garcia del Muro *et al.,* 2005).

(B) Emerging evidence suggests LMS exhibits defects in HR and harbors BRCAness: The relevance of DDR pathways in sarcoma has received little attention until recently. A whole exome and transcriptomic sequencing study of 49 LMS patients, including uterine and non-uterine cases, was recently published in Nature, reflecting the most comprehensive genomic characterization of LMS to date (Chudasama *et al*., 2018). Recurrent mutations were found in *TP53* (49%), *RB1* (27%) and *ATRX* (24%). Approximately 78% of LMS samples demonstrated an alternative lengthening of telomeres phenotype. In this study, deleterious alterations in HR pathway components previously described as synthetic lethal in the setting of PARPi were very common: *PTEN* (57%), *BRCA2* (53%), *FANCA* (27%) *ATM* (22%), *CHEK1* (22%), *XRCC3* (18%), *CHEK2* (12%), *BRCA1* (10%), *RAD51* (10%), *FANCD* (10%). Indeed, enrichment of the Alexandrov-COSMIC mutation signature AC3 (associated with defective HR repair) was found in 98% of LMS samples and the confidence interval of the exposure to AC3 excluded zero in 57%. Evidence of HR defects was observed in both uterine and non-uterine cases. The authors also performed clongenic assays with LMS cell lines (SK-UT-1B, SK-UT-1, SK-LMS-1 and MES-SA) and found these cell lines contained multiple alterations in HR genes and responded to olaparib in a dose dependent fashion. The authors concluded that most LMS tumors exhibit BRCAness and that "genomics-guided clinical trials in LMS patients will be necessary to formally establish whether a BRCAness phenotype confers sensitivity to [PARPi] as in breast, ovarian and prostate cancers." Our proposed study would be the first such evaluation.

Other recently reported genomic sequencing studies reinforce the hypothesis that LMS harbors a BRCAness phenotype. In a study presented at the American Society for Clinical Oncology (ASCO) meeting in 2016, LMS harbored the highest frequency of mutations in HR pathway components with alterations found in *BRCA2*, *ATM*, *ATRX* and others (Italiano *et al.,* 2017; Gounder *et al.*, 2017). In another targeted sequencing study of 54 LMS patients (mostly uterine), mutations in *PALB2* and *ATRX* were seen in 11% and 17%, respectively (Yang *et al.,* 2015).

The *ATRX* protein is of particular interest in sarcoma. In the recently published TCGA sarcoma analysis, *TP53*, *RB1* and *ATRX* were the only recurrently mutated genes observed in LMS (Cancer Genome Atlas Research Network, 2017). In LMS, *ATRX* is mutated in 26-32% of cases but reduced expression as detected by immunohistochemistry is present in up to 50% (Mäkinen *et al.,* 2016; Soumerai *et al.,* 2015). As targeted therapies for *TP53* and *RB1* mutant tumors are non-existent, the identification of novel treatments for *ATRX* mutant sarcomas would be of major therapeutic relevance. *ATRX* is part of the SWI/SNF2 family of chromatin-remodeling proteins and functions in maintenance of genomic stability through modification of heterochromatin. *ATRX* has also been shown to localize to sites of DNA damage and associate with the MRE11-RAD50-NBS protein complex which is critical for repairing DSBs and restarting stalled replication forks (Nandakumar *et al.,* 2017). Indeed, in one recent study, *ATRX* appears necessary for protection of stalled replication forks and, when *ATRX* is lost, compensatory PARP activation is observed, suggestive of a mechanism whereby *ATRX* deficient cancer cells are reliant on PARP (Huh *et al.*, 2016). Indeed, very recent literature suggests *ATRX* deficient tumors have markedly enhanced susceptibility to DNA damaging agents including PARPi (Nandakumar *et al.,* 2017). In one study, patient-derived pediatric glioma cell cultures were

exposed to more than 400 chemotherapies and targeted small molecular inhibitors, and synthetic lethality was observed for *ATRX* mutants treated with the PARP inhibitors olaparib, rucaparib and talazoparib, and these findings were confirmed using CRISPR/Cas9-engineered *ATRX* knockouts (Fazal-Salom *et al.,* 2017). Given the frequency of *ATRX* mutations observed in LMS and the lack of effective targeted therapies of this disease, further evaluation of PARPi for *ATRX* mutant LMS seems warranted.

2.4.1 Preclinical Data

Olaparib has limited activity in sarcoma cell lines as monotherapy, but the combination of olaparib + TMZ has significant anti-proliferative effects in sarcoma cell lines: Olaparib was first tested as monotherapy in a range of sarcoma cell lines. However, in a 5 day dose-response assay in LMS (SK-LMS, SK-UT1, SK-UT1b), malignant peripheral nerve sheath tumor (MPNST, ST8814), and liposarcoma (LS141) cell lines, olaparib had limited activity as monotherapy, with IC_{50} for cell viability exceeding 1000 nM in all cell lines tested (Figure 1). Note, of the LMS cell lines, both SK-UT1 and SK-UT1b are derived from uterine LMS, and SK-LMS is derived from vulvar LMS.

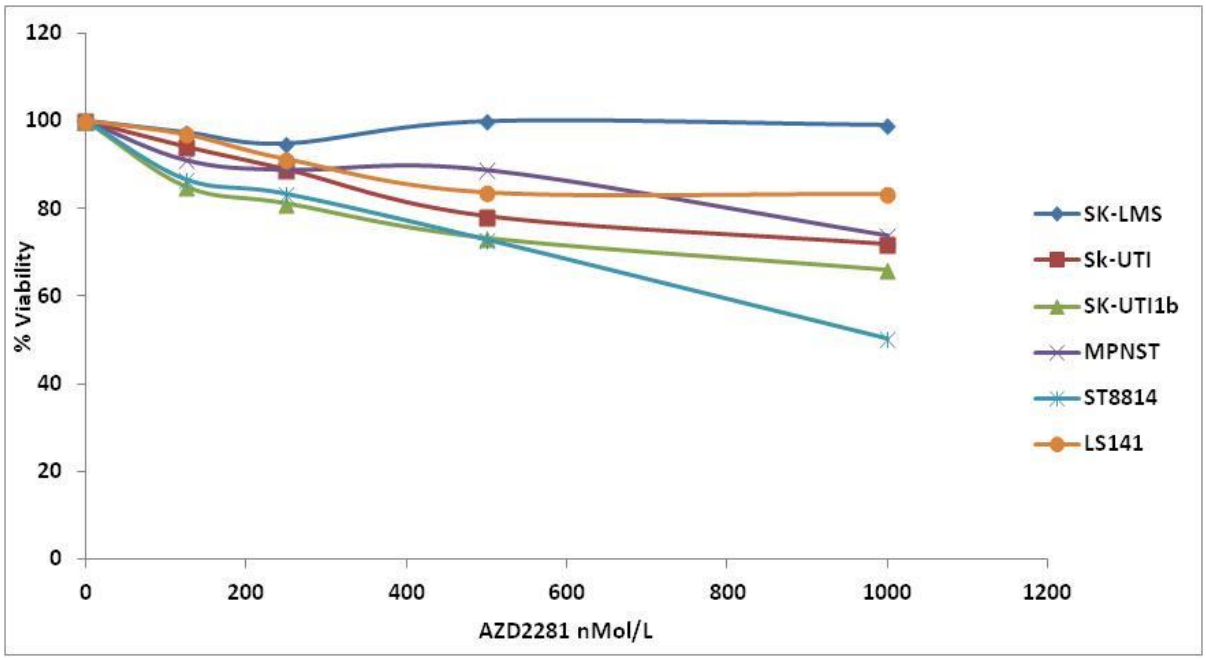

Figure 1: Olaparib (AZD2281) as a single agent has limited anti-proliferative effect on sarcoma cell lines. Cell lines from different sarcoma subtypes were treated with olaparib at doses from 200 – 1000 nmol/L daily for 5 days and cell viability was assessed. A colorimetric cell proliferation assay was used to quantify the amount of formazan dye generated by the activity of dehydrogenases in the cells which is directly proportional to the number of living cells.

Subsequently, combinations of olaparib with chemotherapy were evaluated. As discussed above, the alkylating agent TMZ is considered standard of care monotherapy for several sarcoma subtypes and appears particularly active in LMS and therefore the TMZ + olaparib combination was chosen for initial evaluation with a focus on LMS.

Monotherapy treatment with either TMZ (50-300 mcM) or olaparib (1 mcM) had very limited antiproliferative activity in LMS cell lines as measured in a 5 day cell viability assay (Figure 2). Interestingly, the SK-LMS cell was sensitive to TMZ monotherapy, likely because SK-LMS harbors MGMT promoter hypermethylation, a known predictive biomarker for TMZ as reported in glioblastoma. The incidence of MGMT promoter methylation in LMS is not well defined.

Despite limited activity with TMZ or olaparib monotherapy, when sarcoma cell lines were treated concurrently with 300 mcM TMZ and 1 mcM olaparib, a profound reduction in cell viability was observed, with 90% or greater reduction in viability observed in all cell lines tested (Figure 2). In subsequent experiments, combinations using lower doses of TMZ (50-100 mcM) showed comparable efficacy as the higher TMZ dose (300 mcM) (Figure 2).

Our initial experiments were conducted using TMZ concentrations at the IC_{50} for monotherapy activity in cell viability assays for sarcoma cell lines. However, subsequent to our work being presented, the reported clinical recommended phase 2 dose (RP2D) for the TMZ + olaparib combination (discussed further below) used 75 mg/m^2 TMZ. Review of the available phase 1 pharmacokinetic data for TMZ suggests 75 mg/m^2 oral dosing corresponds to human plasma concentrations of at least 5.6 mcg/mcL (Baruchel *et al.*, 2006; Dhodapkar *et al.*, 1997; Jen *et al.*, 2000; Hammond *et al.*, 1999). Using the molar weight for TMZ (194 g/mol), we estimate this corresponds to plasma concentrations of at least 28 mcM, which is lower than the TMZ dosing used in the preclinical studies (in contrast, the 200 mg twice daily olaparib dose from the RP2D achieves considerably higher human plasma concentrations of approximately 11 mcM). We have conducted additional preclinical experiments showing the combination effect persists using TMZ concentrations as low as 25 mcM (the lowest concentration of TMZ yet evaluated) (Figure 2A). These findings suggest efficacy is conserved even at lower TMZ dose levels and could only be enhanced by the higher olaparib concentrations which would be achieved clinically. These observations are consistent with other preclinical studies of similar combinations (conducted subsequent to ours) suggesting a relatively small amount of DNA damage is sufficient to potentiate the activity of PARP inhibitors and may minimize clinical toxicity, and these considerations likely account for the dose rationale and schedule in the published lung cancer study (Brenner *et al.*, 2012).

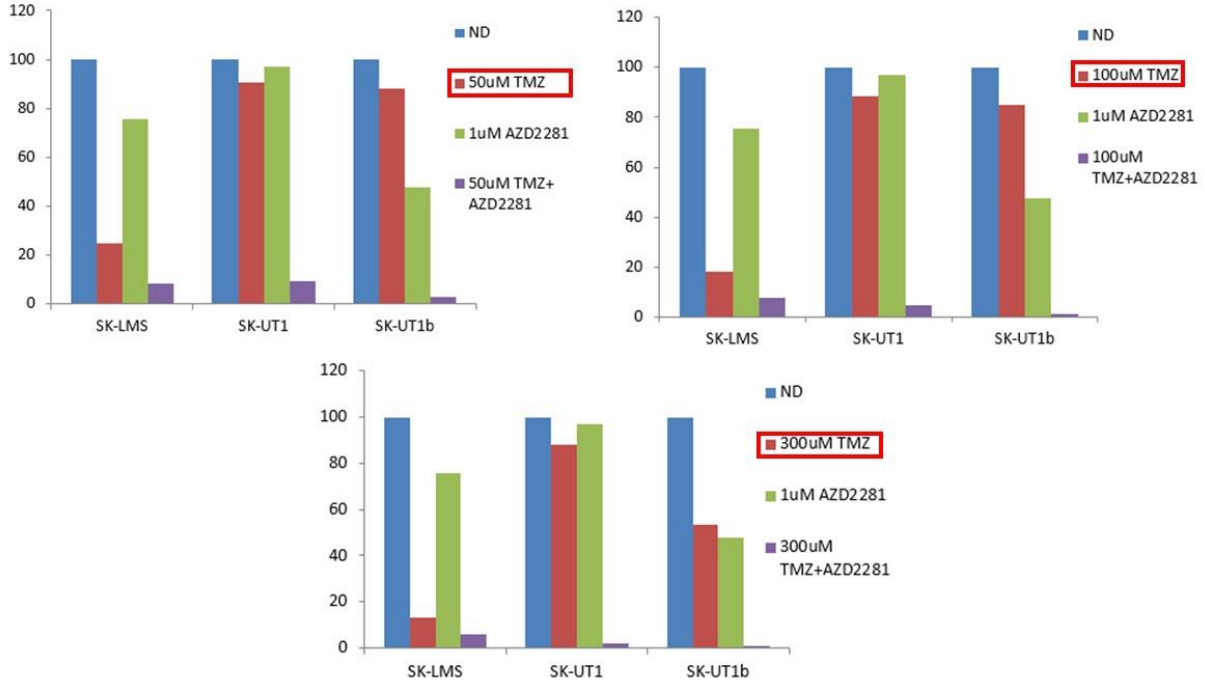

Figure 2: Growth inhibition of sarcoma cell lines is markedly enhanced with combination of olaparib (AZD2281) + TMZ as compared either monotherapy. Cell lines from different sarcoma subtypes were treated with TMZ (50-300 um), olaparib (1 um) or the combination. Drug treatment was for 5 days. Methodology for viability assays is described in Figure 1.

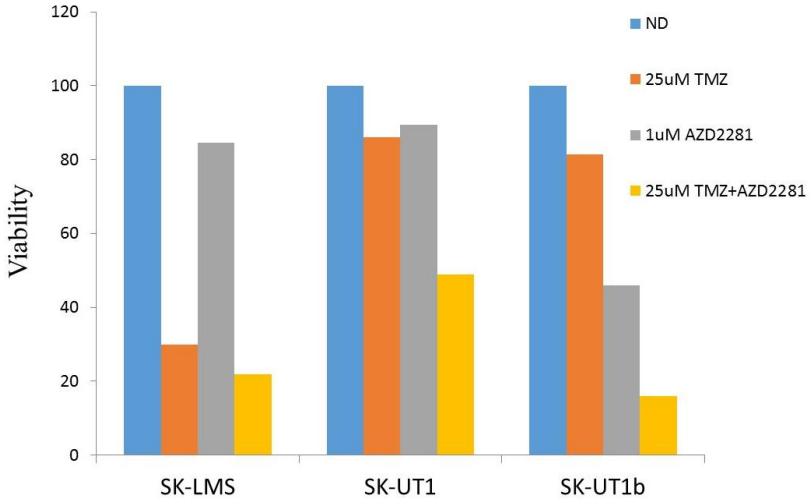

Figure 2A: Growth inhibition of LMS sarcoma cell lines is enhanced with combination of olaparib (AZD2281) + TMZ as compared either monotherapy. Cell lines from different sarcoma subtypes were treated with TMZ (25 um), olaparib (1 um) or the combination (x-axis) and cell viability (y-axis) was assessed (per methods in Figure 1). Drug treatment was for 5 days.

Combination treatment with olaparib + TMZ induces tumor cell DNA damage and apoptosis: Western blot studies were performed to evaluate the mechanism of combination treatment with olaparib + TMZ (Figure 3). Although TMZ or olaparib monotherapy induced

some degree of DNA damage in SK-LMS or SK-UT1b cell lines as measured by phosphorylated H2AX (pH2AX), combination treatment led to markedly greater induction of DNA damage. This enhanced DNA damage was associated with greater induction of p53 and p21 (data not shown) likely reflecting the cellular response to increasing DNA damage. Treatment with olaparib effectively suppressed PAR levels confirming the drug's mechanism and supporting the hypothesis that PARPi in the presence of TMZ led to unrepaired DNA damage and ultimately apoptosis. Critically, in each of the LMS cell lines, the combination treatment effectively induced apoptosis as evidenced by cleaved PARP, whereas little or no evidence of apoptosis was observed with either monotherapy. Similar findings were observed when lower doses of TMZ (50-100 mcM) were employed.

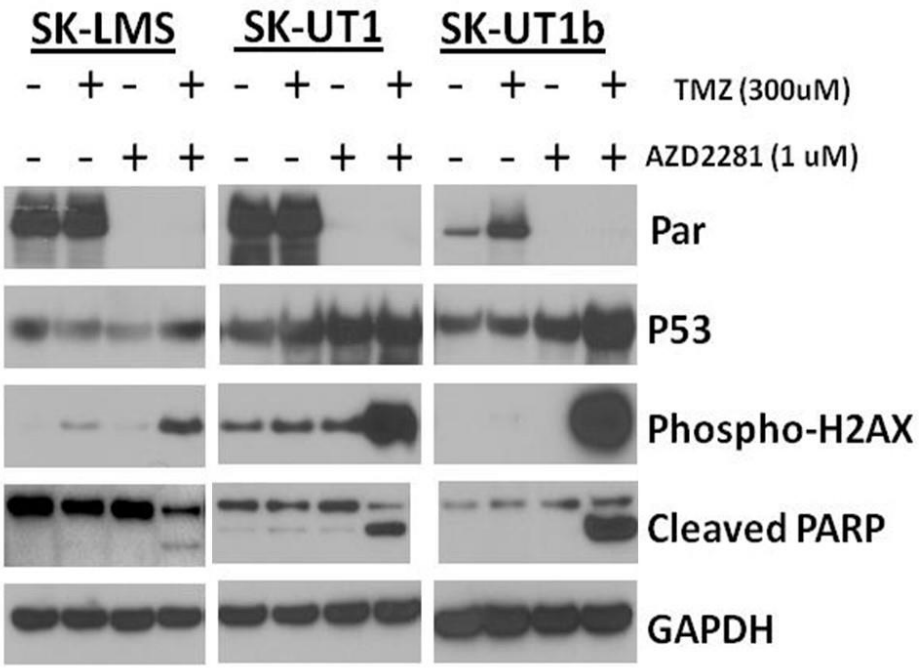

Figure 3: Olaparib (AZD2281) + TMZ treatment promotes DNA damage and induces apoptosis in LMS cell lines. LMS cell lines were treated with no drug (-/-), TMZ (300 mcM), olaparib (AZD2281) (1 mcM) or the combination for 48 hours. Cells were lysed and Western blot analyses were performed for PAR, total p53 and phospho-γH2AX, cleaved PARP and GAPDH as loading control.

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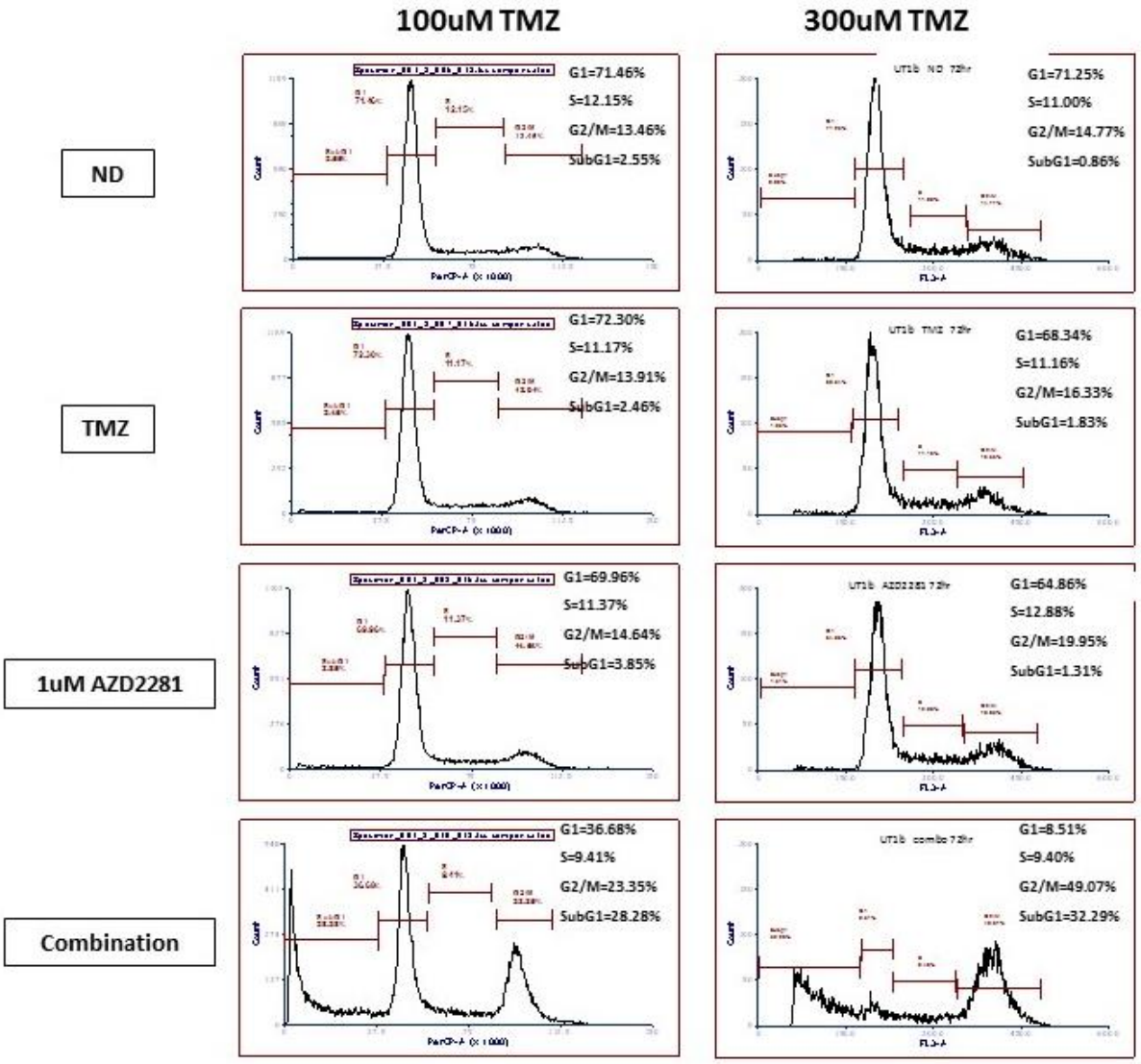
SK-UT1b



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		100 uM TMZ				300 uM TMZ			
		ND	TMZ	AZD2281	COMBO	ND	TMZ	AZD2281	COMBO
SK-UT1	G1	53.5	51.6	50.3	28.5	61.9	59.7	55.5	8.5
	S	18.9	20.8	21.5	15.3	14.1	17.2	16.5	7.8
	G2/M	16.1	21.1	21.6	21.2	18.3	19.7	23.3	59.2
	SubG1	5.5	6.3	6.5	***32.7***	1.5	3.2	2.1	***23.3***
SK-UT1b	G1	71.4	72.3	69.6	36.7	71.3	68.3	64.9	8.5
	S	12.1	11.2	11.4	9.4	11	11.2	12.9	9.4
	G2/M	13.5	13.9	14.6	23.4	14.8	16.3	20	49.1
	SubG1	2.5	2.5	3.9	***28.3***	0.9	1.8	1.3	**32.3**

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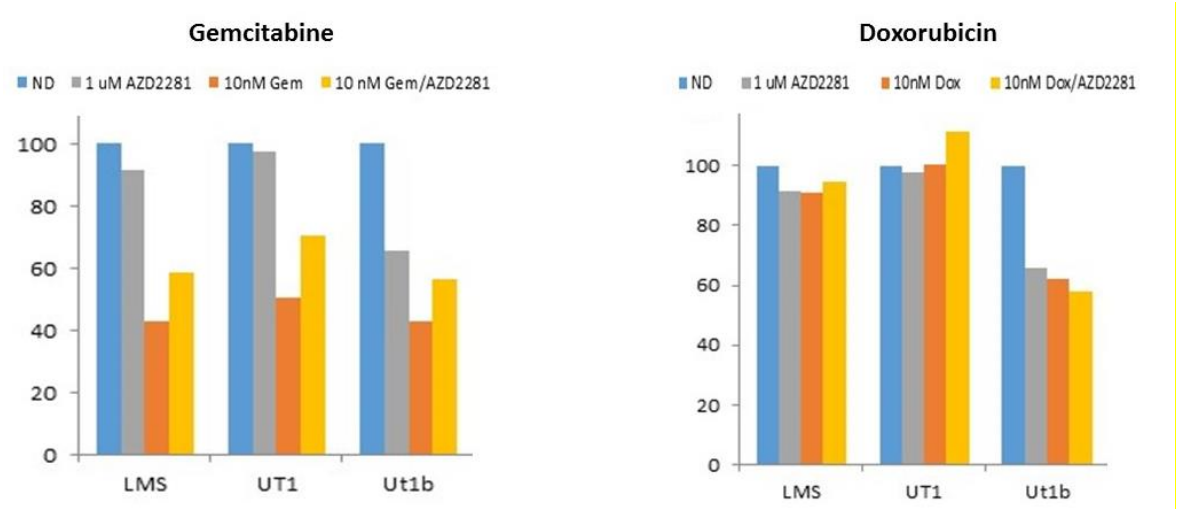

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On the basis of these *in vitro* findings, we attempted to evaluate olaparib + TMZ treatment in mouse xenograft models of SK-UT1b and SK-UT1. In schedule 1 (SK-UT1b xenograft), animals were treated with vehicle control, olaparib 10 mg/kg PO QD, TMZ 50 mg/kg PO QD or olaparib 10 mg/kg + TMZ 50 mg/kg PO QD (all treatments for 3 weeks). In schedule 2 (SK-

UT1 xenograft) animals were treated with olaparib 10 mg/kg PO BID for 5 of 7 days weekly, TMZ 100 mg/kg PO twice per week, or olaparib 10 mg/kg PO BID for 5 of 7 days weekly + TMZ 100 mg/kg PO twice per week. However, both schedules were associated with animal weight loss of 20-25% precluding further evaluation *in vivo*. The toxicity observed in the preclinical in vivo studies may relate to the relatively high doses of TMZ employed as compared to those ultimately evaluated in the clinic.

2.4.2 Clinical Data and Rationale for Dose Selection

The combination of olaparib + TMZ is being tested clinically in two settings: small cell lung cancer (SCLC) and Ewing sarcoma (ES). Both studies have shown favorable tolerability for the combination in heavily pre-treated patients. The phase 1 SCLC study used a 3 + 3 design and results were recently reported at the 2017 American Association for Cancer Research (AACR) Annual Meeting (Farago *et al.*, 2017). The RP2D was TMZ 75 mg/m^2 once daily with olaparib 200 mg twice daily on days 1-7 of a 21-day cycle. No dose limiting toxicities or grade 4/5 adverse events were observed. The most common grade 3 toxicities were neutropenia (38%), anemia (15%) and thrombocytopenia (15%). The overall response rate was 46% and median PFS was 5.6 months which compares favorably with second line chemotherapy in SCLC. The phase 1 study in ES has not been formally reported; however, per our personal communication with the study's principal investigator (Dr. Edwin Choy of Massachusetts General Hospital), the same RP2D and schedule were determined, and no problematic toxicities occurred. These findings suggest that olaparib + TMZ is tolerable in human subjects.

2.5 Correlative Studies Background

2.5.1 Integrated Studies

2.5.1.1 Genomic Alterations In HR Pathway Genes

PARPi and PARPi combination therapies are currently being explored in several advanced solid tumor malignancies. Although clinical development initially focused on tumors with germline BRCA1 and BRCA2 mutations, we now recognize a larger subset of tumors, including LMS, harbor a "BRCAness" phenotype and may therefore be sensitive to this therapeutic approach. BRCAness has been defined as "a phenocopy of BRCA1 or BRCA2 mutation describing the situation in which an HR defect exists in a tumor in the absence of a germline BRCA1 or BRCA2 mutation" (Lord and Ashworth, 2016).

Several approaches to identifying BRCAness have been proposed. Identification of genomic alterations in HR pathway components other than BRCA1 and BRCA2 is perhaps most advanced. Genomic alterations in components of the HR pathway have been evaluated as predictive biomarkers for response to PARPi in several preclinical and clinical studies. Attention initially focused on BRCA-deficient tumors. BRCA deficiency imparts greater than 1,000-fold increase in sensitivity to PARPi in a range of tumor models (Farmer *et al.,* 2005; Bryant *et al.*, 2005). In a phase 1 clinical study of olaparib in 60 solid tumor patients, Response Evaluation Criteria in Solid Tumors (RECIST) objective responses were seen only among the 22 patients with BRCA mutations (9 responded) where the clinical benefit rate (radiologic or tumor-marker

response or meaningful disease stabilization >4 months) was 63% (Fong *et al.,* 2009). In a phase 2 study enrolling endometrial and triple-negative breast cancer patients, the response rate was doubled among the cohort with BRCA mutations as compared those without (Gelmon *et al.,* 2011). In another phase 2 study, favorable response rates were seen in patients with gBRCA1/2 mutations with ovarian, breast, pancreatic and prostate cancers treated with olaparib (Kaufman *et al.,* 2015). In a randomized study of olaparib versus placebo as maintenance therapy in platinum-sensitive recurrent ovarian cancer, considerably greater PFS benefit was seen in patients with germline or somatic BRCA mutations as compared those without such alterations (BRCA-mutant: 11 versus 4 months; hazard ratio [HR], 0.18; 95% confidence interval [CI], 0.10-0.31; non-BRCA mutant: 7 versus 5.5 months; HR, 0.54; 95% CI, 0.34-0.85) (Matulonis *et al.,* 2016). A post-hoc analysis suggested survival benefit from olaparib maintenance was limited to those patients with BRCA mutations.

Several preclinical studies across a range of tumor models suggest that mutations in other components of HR, particularly *ATM*, *ATR*, *CHK1*, *CHK2*, *RAD51*, *PALB2*, *NBS1*, *FANCD2*, *FANCA,* and *FANCC*, among others, predict sensitivity to PARPi monotherapy (Murai *et al.,* 2012; McCabe *et al.,* 2006; Michels *et al.,* 2014; O'Kane *et al.,* 2017). Lord and Ashworth (2016) provides a detailed discussion of the biologic function of each gene in the HR pathway and potential role as biomarker for PARPi treatment. In a phase 2 study of olaparib in metastatic castration-resistant prostate cancer, pre-treatment tumor tissue was assessed for homozygous deletions or deleterious mutations in genes reported to be involved in HR or sensitivity to PARPi including: *BRCA1/2*, *ATM*, *FANCA*, *CHECK2*, *PALB2*, *HDAC2*, *RAD51*, *MLH3*, *ERCC3*, *MRE11,* and *NBS1* (Mateo *et al.,* 2015). Overall, 33% of patients harbored an alteration, most commonly in *BRCA2* and *ATM*. Patients with an aberration in the HR gene panel had a significantly higher response rate to olaparib as compared those who did not (86% versus 6%). Durable clinical responses were observed in patients with *BRCA1/2*, *ATM*, *PALB2*, *FANCA,* and *HDAC* alterations.

More recently, alterations in *ATRX* have been implicated in PARPi sensitivity, which is particularly notable given recurrent mutations in *ATRX* found in LMS (Cancer Genome Atlas Research Network, 2017). *ATRX* is part of the SWI/SNF2 family of chromatin-remodeling proteins and functions in maintenance of genomic stability through modification of heterochromatin. *ATRX* has also been shown to localize to sites of DNA damage and associate with the MRE11-RAD50-NBS protein complex which is critical for repairing DSBs and restarting stalled replication forks (Nandakumar *et al.,* 2017). Indeed, in one recent study, *ATRX* appears necessary for protection of stalled replication forks and when *ATRX* is lost compensatory PARP activation is observed, suggestive of a mechanism whereby ATRX deficient cancer cells are sensitive to PARPi (Huh *et al.,* 2016). Indeed, very recent literature has suggested that *ATRX* deficient tumors have markedly enhanced susceptibility to DNA damaging agents including PARPi (Nandakumar *et al.,* 2017; Watson *et al.*, 2015). In one study, patient-derived pediatric glioma cell cultures were exposed to more than 400 chemotherapies and targeted small molecular inhibitors, and synthetic lethality was observed for ATRX mutants treated with the PARP inhibitors olaparib, rucaparib and talazoparib, and these findings were confirmed using CRISPR/Cas9-engineered *ATRX* knockouts (Fazal-Salom *et al.,* 2017).

Several ongoing clinical trials are evaluating "HR deficiency" gene panels. For example, in an ongoing phase 1 study of niraparib plus carboplatin in patients with HR-deficient advanced solid tumors (NCT03209401), patients are eligible based on biallelic loss or deleterious mutations, as detected by NGS, in the following genes: *ARID1A*, *ATM*, *ATRX*, *MRE11A*, *NBN (NBS1)*, *PTEN*, *RAD50/51/51B*, *BARD1*, *BLM*, *BRCA1*, *BRCA2*, *BRIP1*, *FANCA/C/D2/E/F/G/L*, *PALB2*, *WRN*, *CHK2*, *CHK1*, *CDKN1A/B/C*, *CDKN2A*, *BAP1*, *FAM175A*, *SLX4*, *MLL2,* or *XRCC*. Weighing the current preclinical and clinical evidence as well as available next generation sequencing data from LMS, we will include *BRCA1*, *BRCA2*, *ATM*, *ATR*, *ATRX*, *CHK1*, *CHK2*, *RAD51*, *PALB2*, *FANCA,* and *NBS1* in our HR panel. A patient will be considered biomarker-positive if a homozygous deletion or deleterious mutation categorized as tier 1-3 is identified in any one of these genes.

2.5.1.2 RAD51 Foci Formation Assay (Functional HR assay)

Several approaches to identifying BRCAness have been proposed. Functional assays of HR repair have emerged as a particularly promising potential biomarker for cancer cell sensitivity to DNA damaging therapies including PARPi. RAD51 plays an essential role in HR by mediating pairing of homologous DNA sequences and strand exchange thus allowing for high-fidelity repair of DNA damage (Baumann and West, 1998). The formation of RAD51 foci after exposure to DNA damaging agents may serve as a functional and more encompassing measure of overall HR activity in the cancer cell (Gachechiladze *et al.,* 2017). Considerable preclinical literature supports RAD51 foci formation as a potential biomarker for PARPi sensitivity. An analysis of seven ovarian cancer cell lines showed those sensitive to PARPi exhibited significantly less RAD51 foci formation after irradiation (Shah *et al.,* 2014). In pancreatic, cervical and lung cancer preclinical models, siRNA knockdown of RAD51 markedly increases sensitivity to DNA damaging agents (Klein, 2008; Ito *et al.,* 2005).

From a clinical perspective, RAD51 foci formation could be evaluated (1) *in vivo* using tumor biopsy specimens obtained after patients receive DNA damaging treatments or (2) *ex vivo* whereby fresh tumor samples obtained at baseline (pre-treatment) are exposed to DNA damage and RAD51 foci formation assessed. In one study, breast cancer patients were treated with neoadjuvant anthracycline-based chemotherapy and biopsies were obtained 24 hours after the first cycle of chemotherapy. RAD51 foci were assessed by immunofluorescence and a RAD51 score was assigned based on the proportion of proliferative cells with RAD51 foci. RAD51 foci formation correlated with complete pathologic response at surgery, as 33% of those with low RAD51 scores ("HR deficient") achieved pathologic complete response as compared 3% of HR proficient group (p=0.01) (Graeser *et al.,* 2010). Similarly, in esophageal cancer patients treated with neoadjuvant DNA-damaging chemotherapy, pathologic complete responses were more common in RAD51 "negative" as compared RAD51 "positive" cases (Nakanoko *et al.,* 2014).

Ex-vivo RAD51 assays have also shown promise. In one study, ascitic fluid cultures from 50 chemotherapy naïve ovarian cancer patients were assessed with a functional RAD51 immunofluorescence assay to classify patients as HR-proficient or HR-deficient. All patients subsequently received platinum-based chemotherapy. HR-deficiency was associated with higher *ex-vivo* PARPi sensitivity and clinical platinum sensitivity (Naipal *et al.,* 2014; Mukhopadhyay *et al.,* 2012). In a study of patient derived xenografts (PDXs) of ovarian cancer, RAD51 foci

formation after exposure to irradiation predicted sensitivity of PDXs to PARP inhibitors *in vitro* and *in vivo* (Shah *et al.,* 2014). Collectively, these data support the further evaluation of RAD51 foci formation as a possible biomarker for sensitivity to PARPi and PARPi combinations. For the purposes of this study, the assay will be applied to the pre-treatment, on-treatment, and optional pre-progression biopsies. This study will evaluate a RAD51 foci assay developed by Dr. Geoffrey Shapiro of the Dana Farber Cancer Institute.

2.5.2 Exploratory Studies

2.5.2.1 MGMT Expression

TMZ is an oral alkylating agent used in the treatment of several cancers, including sarcoma, for which this agent is listed on the National Comprehensive Cancer Network guidelines. TMZ treatment results in methylation at several DNA sites, including N^7-methylguanine, N^3-methylguanine, and O^6-methylguanine. The first two alterations are associated with low cytotoxic potential because these adducts are efficiently repaired by base excision repair. O^6-methylguanine is responsible for most of the cytotoxicity induced by TMZ. This abnormal base pair stalls the DNA replication fork triggering a DNA damage response. The O^6-methylguanine DNA methyltransferase (MGMT) gene encodes the DNA repair enzyme that removes the methyl group from O^6 guanine thereby efficiently repairing DNA damage imparted by TMZ (Thomas *et al.,* 2013). Expression of the MGMT repair enzyme is epigenetically silenced in several tumor types by promoter hypermethylation. These cancer cells cannot repair TMZ-induced DNA damage and undergo programmed cell death upon TMZ treatment. In contrast, high levels of MGMT expression, which allow repair of TMZ-induced DNA damage, have been correlated with resistance to alkylating agents in numerous preclinical models (Hegi *et al.,* 2008).

MGMT promoter methylation has been evaluated as a biomarker for response to TMZ in glioblastoma, where TMZ is standard of care in the adjuvant and metastatic treatment settings. The effects of MGMT promoter methylation on clinical benefit from TMZ were evaluated retrospectively using tumor specimens from a phase 2 randomized study of adjuvant radiotherapy versus adjuvant radiotherapy + TMZ following definitive resection of glioblastoma (Hegi *et al.,* 2005). Using methylation-specific PCR, 45% of cases were considered to have MGMT promoter methylation which was associated with prolonged survival from TMZ treatment. In a separate study, MGMT promoter methylation was associated with higher objective response rates to TMZ in advanced, recurrent glioblastoma (Paz *et al.,* 2004). Retrospective clinical studies have suggested MGMT promoter methylation predicts for increased clinical benefit from TMZ in pancreatic neuroendocrine tumors, melanoma, colorectal cancer and other tumor types (Campana *et al*., 2018; Sartore-Bianchi *et al.,* 2017; Tuominen *et al.,* 2015). In addition, reexpression of the MGMT enzyme by several means, including epigenetic modification, has been described as a mechanism of resistance to TMZ monotherapy, and therefore reevaluating the status of MGMT protein expression in the on-treatment and pre-progression biopsies is also of interest (Zhang *et al.,* 2012).

2.5.2.2 SLFN11 Expression

Schlafen family member number 11 (SLFN11) is an emerging biomarker for defective HR. Expression of SLFN11 is a dominant determinant of sensitivity to DNA damaging agents including PARPi in several preclinical cancer models although the mechanism has not been fully elucidated (Murai *et al.,* 2016). Replication protein A (RPA) is a protein complex which binds single stranded DNA (ssDNA) and functions to recruit various enzymes involved in maintenance and repair of DNA. SLFN11 may inhibit HR by removing RPA from ssDNA. SLFN11 may also function as a cell cycle checkpoint upstream of ATM and ATR preventing cell cycle progression in the setting of DNA damage (Ballestrero *et al.,* 2017). High SLFN11 levels have been proposed to confer a BRCA-like state of HR deficiency and SLFN11 is considered among the most promising biomarkers for DNA damaging agents, but SLFN11 has received limited evaluation in prospective clinical trials (Mu *et al.,* 2016).

In an analysis using the NCI-60 cell line panel, responsiveness of cancer cells to PARPi and PARPi + TMZ combinations strongly correlated with expression of SLFN11 (Murai *et al.,* 2016). In SCLC cell lines, loss of SLFN11 confers resistance to PARPi, while SLFN11 transcript and protein expression correlate directly with PARPi sensitivity (Lok *et al.,* 2017). In SCLC PDX models, SLFN11 expression by immunohistochemistry was strongly associated with response to both PARPi monotherapy and PARPi + TMZ combinations (Lok *et al.,* 2017). SLFN11 is highly expressed in Ewing sarcoma (ES), desmoplastic small round cell tumor (DSRCT), osteosarcoma and rhabdomyosarcoma and correlates with response to PARPi and ionizing radiation in those malignancies (Shelat *et al.,* 2017). In a preclinical study in ES, the characteristic EWS-FLI1 fusion protein was shown to regulate SLFN11 expression and SLFN11 knockdown imparted resistance to combination treatment with PARPi + TMZ (Tang *et al.,* 2015; Stewart *et al.*, 2014).

3. PATIENT SELECTION

3.1 Eligibility Criteria

3.1.1 Patients must have histologically documented LMS of uterine origin. Pathology review and confirmation of diagnosis will occur at the site enrolling the patient on this study.

3.1.2 Patients must have locally advanced and unresectable or metastatic disease.

3.1.3 Patients must have disease which is measurable at study entry according to RECIST version 1.1 criteria. Additionally, patients must have a site of disease deemed accessible for biopsy at no or minimal risk to the patient (including through the use of image-guidance). If there are questions regarding the feasibility of biopsy, the case should be reviewed with interventional radiology or the appropriate department at the study site prior to registration.

3.1.4 Patients must have had prior progression on, or intolerance to, at least one line of systemic therapy for advanced LMS. Adjuvant therapy administered after curative resection will not qualify as prior treatment. There is no upper limit on the number of

prior therapies received.

3.1.5 Patients must be ≥18 years of age. Uterine LMS affects older adults and is rarely encountered in children and adolescents.

3.1.6 Patients must demonstrate an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of ≤2 (Karnofsky ≥50%, see Appendix A).

3.1.7 Patients must have normal organ and bone marrow function measured within 14 days prior to administration of study treatment as defined below:

– absolute neutrophil count	≥1,500/mcL
– hemoglobin	≥9 g/dL*
– platelets	≥100,000/mcL
– total bilirubin	≤1.5 × institutional upper limit of normal (ULN)
– AST(SGOT)/ALT(SGPT)	≤2.5 × institutional ULN
– glomerular filtration rate (GFR)	≥51 mL/min, based on a 24-hour urine test for creatinine clearance or estimated using the Cockcroft-Gault equation of:

$$\text{Estimated GFR} = \frac{(140\text{-age [years]}) \times \text{weight (kg)}}{\text{serum creatinine (mg/dL)} \times 72} \quad (\times F)^{a}$$

[a] where F=0.85 for females and F=1 for males.

* Without transfusion of packed red blood cells within the past 28 days

3.1.8 If patients have evidence of chronic hepatitis B virus (HBV) infection, HBV viral load must be undetectable on suppressive therapy if indicated. Patients with a past or resolved HBV infection (defined as the presence of hepatitis B core antibody and absence of HBsAg) are eligible.

3.1.9 If patients have a history of hepatitis C virus (HCV) infection, they must be treated with undetectable HCV viral load (polymerase chain reaction is negative for HCV RNA).

3.1.10 Patients must be postmenopausal or have evidence of non-childbearing status, OR, for women of childbearing potential, must have a negative urine or serum pregnancy test within 28 days of study treatment and confirmed again on Day 1 prior to study treatment.

Postmenopausal is defined as:

- Amenorrhoeic for ≥ 1 year following cessation of exogenous hormonal treatments
- Luteinizing hormone (LH) and follicle stimulating hormone (FSH) levels in the postmenopausal range for women under 50
- radiation-induced oophorectomy with last menses >1 year ago
- chemotherapy-induced menopause with >1 year interval since last menses, or

- surgical sterilization (bilateral oophorectomy or hysterectomy)

3.1.11 Patients and their partners, if sexually active and of childbearing potential, must agree to the use of two highly effective forms of contraception in combination throughout the period of taking study treatment and for 3 months after the last dose of study drug(s) to prevent pregnancy in the study patient or partner.

3.1.12 Patients must be able to swallow orally administered medication.

3.1.13 Patients must have a life expectancy ≥16 weeks.

3.1.14 Patients must be able to understand and be willing to sign a written informed consent document. Patients with impaired decision-making capacity (IDMC) will be eligible if they have a close caregiver or legally authorized representative (LAR) available to assist them.

3.1.15 Patients must be willing and able to comply with the protocol for the duration of the study, including undergoing treatment and attending scheduled visits and examinations.

3.1.16 Patients with HIV infection may be enrolled on this study provided: (a) they are on a stable regimen of highly active anti-retroviral therapy (HAART) with no medications otherwise prohibited by this protocol (e.g. drug-drug interactions) and (b) require no concurrent antibiotics or antifungals for the prevention of opportunistic infections and (c) have a CD4 count above 250 cell/mcL and an undetectable viral load on standard PCR-based tests within 1 month of initiation of study treatment. Other patients with clinically significant immunosuppression, *e.g*. organ transplant patients, are not eligible. If clarification is needed, this may be discussed with the medical monitor.

3.1.17 Patients must be able to have temozolomide provided as a standard of care medication.

3.2 Exclusion Criteria

3.2.1 Patients must not have had any previous treatment with any poly(adenosine diphosphate[ADP]-ribose) polymerase (PARP) inhibitors, including olaparib, or prior treatment with dacarbazine and/or temozolomide.

3.2.2 Patients must have recovered from adverse events due to prior anti-cancer therapy (*i.e.*, may not have residual toxicities > grade 1 or above baseline), excluding alopecia. Patients who have endocrinopathies associated with prior immunotherapy treatment but which are controlled with replacement therapy are eligible.

3.2.3 Prior to initiating study treatment, at least 28 days must have elapsed from the last dose of systemic anti-cancer treatment (cytotoxic, biologic or immunotherapeutic) or radiation therapy (except for palliative radiation, in which case a 14-day washout applies).

3.2.4 Patients must not have had major surgery within 2 weeks of starting study treatment and must have recovered from any effects of any major surgery that occurred >2 weeks before starting study treatment.

3.2.5 Patients must not be receiving any other investigational agent.

3.2.6 Patients must not have been diagnosed with another malignancy unless curatively treated with no evidence of disease for ≥5 years except: adequately treated non-melanoma skin cancer, curatively treated in situ cancer of the cervix, ductal carcinoma in situ (DCIS), or any other malignant condition considered indolent and unlikely to require active therapy.

3.2.7 Patients must not have myelodysplastic syndrome (MDS)/acute myeloid leukemia (AML) or bone marrow biopsy findings consistent with MDS and/or AML.

3.2.8 Patients must not have active central nervous system (CNS) or leptomeningeal disease at the time of enrollment. Patients with a history of such disease previously treated with curative intent (such as with surgery or radiation) that have not progressed on subsequent imaging, have been clinically asymptomatic, and have not received systemic corticosteroids for at least 28 days, are eligible.

3.2.9 Patients must not have a history of allergic reactions attributed to compounds of similar chemical or biologic composition to olaparib or TMZ or any of the excipients of any study product.

3.2.10 Patients must refrain from concomitant use of known strong CYP3A inhibitors (*e.g.*, itraconazole, telithromycin, clarithromycin, protease inhibitors boosted with ritonavir or cobicistat, indinavir, saquinavir, nelfinavir, boceprevir, telaprevir) or moderate CYP3A inhibitors (*e.g.,* ciprofloxacin, erythromycin, diltiazem, fluconazole, verapamil). The required washout period for strong or moderate CYP3A inhibitors prior to starting olaparib is 2 weeks.

Because the lists of these agents are constantly changing, it is important to regularly consult a frequently-updated medical reference. As part of the enrollment/informed consent procedures, the patient will be counseled on the risk of interactions with other agents, and what to do if new medications need to be prescribed or if the patient is considering a new over-the-counter medicine or herbal product.

3.2.11 Patients must refrain from concomitant use of known strong CYP3A inducers (*e.g.*, phenobarbital, enzalutamide, phenytoin, rifampicin, rifabutin, rifapentine, carbamazepine, nevirapine and St John's Wort) or moderate CYP3A inducers (*e.g.*, bosentan, efavirenz, modafinil). The required washout period for strong or moderate CYP3A inducers prior to starting olaparib is 5 weeks for enzalutamide or phenobarbital and 3 weeks for other agents.

3.2.12 Patients must not have an uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, uncontrolled ventricular arrhythmia, recent (within 3 months)

myocardial infarction, uncontrolled major seizure disorder, unstable spinal cord compression, superior vena cava syndrome, extensive interstitial bilateral lung disease on high resolution computed tomography (HRCT) scan, or psychiatric illness that would limit compliance with study requirements.

3.2.13 Pregnant women are excluded from this study because olaparib is a PARP inhibitor with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with olaparib, breastfeeding should be discontinued if the mother is treated with olaparib. These potential risks may also apply to other agents used in this study.

3.2.14 Patients must not have gastrointestinal disorders likely to interfere with absorption of the study medication.

3.2.15 Patients must not have had involvement in the planning and/or conduct of the study.

3.3 Inclusion of Women and Minorities

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

4. REGISTRATION PROCEDURES

4.1 Investigator and Research Associate Registration with CTEP

Food and Drug Administration (FDA) regulations require Investigational New Drug (IND) sponsors to select qualified investigators. National Cancer Institute (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 Cancer Therapy Evaluation Program (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 Oncology Patient Enrollment Network (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 Cancer Trials Support Unit (CTSU) 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 Principal Investigator (PI) on the Institutional Review Board (IRB) approval

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.

4.2 Site Registration

This study is supported by the NCI CTSU.

Each investigator or group of investigators at a clinical site must obtain IRB approval for this protocol and submit IRB approval and supporting documentation to the CTSU Regulatory Office before they can be approved to enroll patients. Assignment of site registration status in the CTSU Regulatory Support System (RSS) uses extensive data to make a determination of whether a site has fulfilled all regulatory criteria including but not limited to the following:

- An active Federalwide Assurance (FWA) number
- An active roster affiliation with the Lead Network or a participating organization
- A valid IRB approval
- Compliance with all protocol specific requirements.

In addition, the site-protocol PI must meet the following criteria:

- Active registration status

- The IRB number of the site IRB of record listed on their Form FDA 1572
- An active status on a participating roster at the registering site

Sites participating on the NCI CIRB initiative that are approved by the CIRB for this study are not required to submit IRB approval documentation to the CTSU Regulatory Office. For sites using the CIRB, IRB approval information is received from the CIRB and applied to the RSS in an automated process. Signatory Institutions must submit a Study Specific Worksheet for Local Context (SSW) to the CIRB via IRBManager to indicate their intent to open the study locally. The CIRB's approval of the SSW is then communicated to the CTSU Regulatory Office. In order for the SSW approval to be processed, the Signatory Institution must inform the CTSU which CIRB-approved institutions aligned with the Signatory Institution are participating in the study.

4.2.1 Downloading Regulatory Documents

Site registration forms may be downloaded from the 10250 protocol page located on the CTSU Web site. Permission to view and download this protocol is restricted and is based on person and site roster data housed in the CTSU RSS. To participate, Investigators and Associates must be associated with the Corresponding or Participating protocol organization in the RSS.

- Go to https://www.ctsu.org and log in using your CTEP-IAM username and password.
- Click on the Protocols tab in the upper left of your screen.
- Either enter the protocol # in the search field at the top of the protocol tree, or
- Click on the By Lead Organization folder to expand, then select LAO-CT018, and protocol #10250.
- Click on LPO Documents, select the Site Registration documents link, and download and complete the forms provided. (Note: For sites under the CIRB initiative, IRB data will load to RSS as described above.)

4.2.2 Requirements For 10250 Site Registration

- IRB approval (For sites not participating via the NCI CIRB; local IRB documentation, an IRB-signed CTSU IRB Certification Form, Protocol of Human Subjects Assurance Identification/IRB Certification/Declaration of Exemption Form, or combination is accepted.)
- For applicable ETCTN studies with a radiation and/or imaging (RTI) component, the enrolling site must be aligned to a RTI provider. To manage provider associations access the Provider Association tab on the CTSU website at https://www.ctsu.org/RSS/RTFProviderAssociation, to add or remove associated providers. Sites must be linked to at least one IROC credentialed provider to participate on trials with an RT component. Enrolling sites are responsible for ensuring that the appropriate agreements are in place with their RTI provider, and that appropriate IRB approvals are in place.

- Site Initiation Visit Teleconference
- ETCTN Specimen Tracking Training with Theradex
  - All data entry users (Clinical Research Associate role) at each participating site will need to complete the Theradex-led training.
  - Theradex will provide a certificate of completion, which will need to be submitted to the CTSU through the Regulatory Submission Portal.
  - The training is a one-time only requirement per individual. If an individual has previously completed the training for another ETCTN study, the training does not need to be completed again nor does the certificate of completion need to be resubmitted to the CTSU. However, new versions of the Specimen Tracking system may require new training.
  - This training will need to be completed before the first patient enrollment at a given site.
  - Peter Clark and Diana Vulih are the main points of contact at Theradex for the training (PClark@theradex.com and DVulih@theradex.com, Theradex phone: 609-799-7580).

4.2.3 Submitting Regulatory Documents

Submit required forms and documents to the CTSU Regulatory Office, where they will be entered and tracked in the CTSU RSS.

Regulatory Submission Portal: www.ctsu.org (members' area) → Regulatory Tab →Regulatory Submission
When applicable, original documents should be mailed to:
CTSU Regulatory Office
1818 Market Street, Suite 3000
Philadelphia, PA 19103

Institutions with patients waiting that are unable to use the Portal should alert the CTSU Regulatory Office immediately at 1-866-651-2878 in order to receive further instruction and support.

4.2.4 Checking **Site** Registration Status

You can verify your site registration status on the members' section of the CTSU website.

- Go to https://www.ctsu.org and log in to the members' area using your CTEP-IAM username and password.
- Click on the Regulatory tab at the top of your screen.
- Click on the Site Registration tab.
- Enter your 5-character CTEP Institution Code and click on Go.

Note: The status given only reflects compliance with IRB documentation and institutional compliance with protocol-specific requirements as outlined by the Lead

Network. It does not reflect compliance with protocol requirements for individuals participating on the protocol or the enrolling investigator's status with the NCI or their affiliated networks.

4.3 Patient Registration

4.3.1 OPEN / IWRS

Patient enrollment will be facilitated using the Oncology Patient Enrollment Network (OPEN). OPEN is a web-based registration system available to users on a 24/7 basis. It is integrated with the CTSU Enterprise System for regulatory and roster data interchange and with the Theradex Interactive Web Response System (IWRS) for retrieval of patient registration/randomization assignment. Patient enrollment data entered by Registrars in OPEN / IWRS will automatically transfer to the NCI's clinical data management system, Medidata Rave.

The OPEN system will provide the site with a printable confirmation of registration and treatment information. Please print this confirmation for your records.

4.3.2 OPEN/IWRS User Requirements

OPEN/IWRS users must meet the following requirements:

- Have a valid CTEP-IAM account (*i.e.*, CTEP username and password).
- To enroll patients or request slot reservations: Be on an ETCTN Corresponding or Participating Organization roster with the role of Registrar. Registrars must hold a minimum of an AP registration type.
- To approve slot reservations or access cohort management: Be identified to Theradex as the "Client Admin" for the study.
- Have regulatory approval for the conduct of the study at their site.

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

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

4.3.3 Special Instructions for Patient Enrollment

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

- All biospecimens collected for this trial must be submitted using the ETCTN Specimen Tracking System (STS) unless otherwise noted.
- The system is accessed through special Rave user roles: "CRA Specimen Tracking" for data entry at the treating institutions and "Biorepository" for users receiving the specimens for processing and storage at reference labs and the Biorepository.
- Please refer to the Medidata Account Activation and Study Invitation Acceptance link on the CTSU website under the Rave/DQP tab.

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

Detailed instructions can be found in Section 5.3.

4.3.4 OPEN/IWRS Questions?

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

4.4 General Guidelines

Following registration, patients should begin protocol treatment within 5 business days. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a patient does not receive protocol therapy following registration, the patient's registration on the study may be canceled. The Study Coordinator should be notified of cancellations as soon as possible.

5. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

5.1 Summary Table for Specimen Collection

Time Point	Specimen	Send Specimens To:
PRE-TREATMENT (after informed consent and within 14 days of Cycle 1 Day 1)		
	• 4-8 formalin fixed cores[1] • 10 mL blood in EDTA tube	ETCTN Biorepository
ON-TREATMENT (C2 D3-5, 2-6 hours post AM olaparib and temozolomide treatment)		
	• 4-6 formalin fixed cores[1]	ETCTN Biorepository
PRE-PROGRESSION (if applicable, see Section 5.4.2)		
	• 4-8 formalin fixed cores[1]	ETCTN Biorepository
[1]For new biopsies, a copy of the radiology and operative reports from the tissue removal procedure must be sent with the tissue to the ETCTN Biorepository. When completed, upload the corresponding pathology reports to Rave.		

5.2 Specimen Procurement Kits and Scheduling

5.2.1 Specimen Shipping Kits

Kits for the collection and shipment of specimens to the ETCTN Biorepository can be ordered online via the Kit Management system: (https://ricapps.nationwidechildrens.org/KitManagement).

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

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

5.2.2 Scheduling of Specimen Collections

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

- Tumor tissue specimens collected during biopsy procedures and fixed in formalin must be shipped on the same day of collection.
- Tissue can be collected Monday through Wednesday and shipped overnight for arrival on Tuesday through Thursday at the ETCTN Biorepository at Nationwide Children's Hospital.
- Fresh blood specimens may be collected and shipped Monday through Friday.

5.3 Specimen Tracking System Instructions

5.3.1 Specimen Tracking System Overview and Enrollment Instructions

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

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

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

Immediately following enrollment, the institutional anatomical pathology report for the diagnosis under which the patient is being enrolled must be uploaded into Rave. The report must include the surgical pathology ID (SPID), collection date, block number, and the IWRS-assigned UPID and patient study ID for this trial. For newly acquired biopsies, the radiology and operative report(s) must also be uploaded into Rave. **Important: Remove any personally identifying information, including, but not limited to, the patient's name, initials, medical record number, and patient contact information from the institutional pathology report prior to submission.**

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

For questions regarding the Specimen Tracking System, please contact the Theradex Help Desk at CTMSSupport@theradex.com.

A shipping manifest **must** be included with all sample submissions.

5.3.2 Specimen Labeling

5.3.2.1 Tissue Specimen Labels

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

- Patient Study ID
- Universal Patient ID (UPID)
- Specimen ID (automatically generated by Rave)
- Time point
- Specimen type (FFPE Block, Formalin Fixed Tissue, Fresh Tissue in Media, *etc.*)
- Tissue type (P for primary, M for metastatic or N for normal)
- Surgical pathology ID (SPID) number
- Block number from the corresponding pathology report (archival only)
- Collection date (to be added by hand)

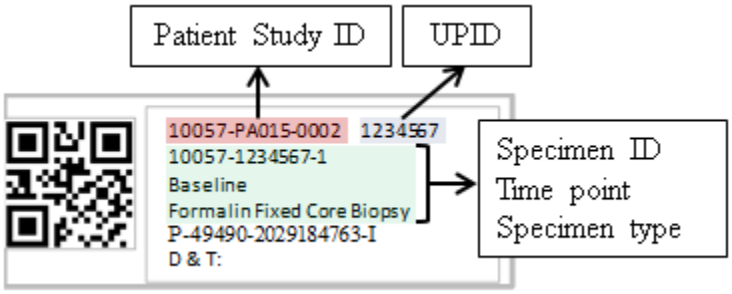
5.3.2.2 Blood Specimen Labels

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

- Patient Study ID
- Universal Patient ID (UPID)
- Specimen ID (automatically generated by Rave)
- Time point
- Specimen type (*e.g.*, blood, serum)
- Collection date (to be added by hand)

5.3.2.3 Example of Specimen Label

The following image is an example of a tissue specimen label printed on a standard Avery label that is 1" high and 2.625" wide.



The QR code in the above example is for the Specimen ID shown on the second line.
NOTE: The QR code label is currently under development at Theradex as of 31-Aug-2018; therefore, labels generated by the STS for this study may not include a QR code.

The second line item from the end includes four data points joined together:

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

The last line on the example label is for the handwritten date and optional time.

5.3.3 Overview of Process at Treating Site

5.3.3.1 OPEN Registration

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

Registration without eligibility specimen analysis:

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

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

5.3.3.2 Rave Specimen Tracking Process Steps

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

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

Step 2: Print labels using report in EDC and collect specimen.

- Label specimen containers and write collection date on each label.
- After collection, store labeled specimens as described in Section 5.3
- Apply an extra specimen label to *each* report before scanning. Return to the **Histology and Disease** form to upload any initial Pathology, Radiology, Molecular Reports (up to 4), Surgical (or Operative) reports and Pathology Verification form (when applicable). Return to **Specimen Tracking Enrollment** CRF to upload any molecular report (one per specimen) and/or specimen specific pathology or related report (one per specimen). Uploaded reports should have protected health information (PHI) data like name, mailing address, medical record number or social security number (SSN) redacted. Do not redact surgical pathology ID (SPID), block number or relevant dates, and include UPID and patient study ID on each document.

Step 3: Complete specimen data entry.

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

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

- **Shipping Status** CRF**:** Enter tracking number, your contact information, recipient, number of containers and ship date once for the 1st specimen in a shipment.
- **Copy Shipping** CRF**:** Select additional specimens to add to an existing shipment referenced by the tracking number.

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

- Print two copies of the shipping list, one to provide in the box, the other for your own records.
- Print pathology or other required reports to include in the box. Be sure the printed copy includes the specimen label.

Step 6: Send email notification.

- For only one of the specimens in the shipment, click “Send Email Alert”

checkbox on the **Shipping Status** CRF to email recipient.

Step 7: Ship the specimen(s).

5.4 Specimen Collection and Processing

5.4.1 Biopsy Collection Procedure

Mandatory core biopsy specimens will be obtained at two time points. The first ("pre-treatment") biopsy will be obtained before beginning study treatment (after informed consent is obtained and up to 14 day prior to cycle 1 day 1). The second ("on-treatment") biopsy will be performed during cycle 2, days 3-5.

A third, optional ("pre-progression") biopsy will be collected from patients who meet certain criteria and provide informed consent for this biopsy (see Section 5.4.2 below).

5.4.2 Optional "Pre-Progression" Tumor Biopsy

The investigators will seek to evaluate the RAD51, MGMT, and SLFN11 assays in patients who have evidence of clinical benefit from the study drug but later show evidence of radiographic progression not meeting RECIST criteria for progressive disease.

To undergo this biopsy, all of the following criteria must be met:

1. The patient has provided informed consent for the optional tumor biopsy, which is part of the informed consent document for this study.

2. The patient has evidence of clinical benefit from study treatment manifest by either:
    a. RECIST complete or partial response within the first 6 months of study treatment
    b. Stable disease, by RECIST criteria, of at least 4 cycles (12 weeks) duration

3. At a subsequent timepoint, the patient has study-specified imaging showing evidence of radiographic disease progression, but which does not meet RECIST criteria for progressive disease (RECIST + 5-19% from the baseline study) and the patient lacks clinical progression, and the investigator intends to continue the study treatment.

When all of these criteria apply, a third ("pre-progression") tumor biopsy will be performed. The same procedures as described in Section 5.4.1 will apply to this biopsy. The biopsy will occur during days 3-5 of the next treatment cycle initiated following the timepoint at which these criteria are met, 2-6 hours post AM olaparib and temozolomide treatment. The same site of disease should be biopsied if at all possible.

If questions arise regarding whether a given patient is considered appropriate for the optional biopsy, the treating investigator should discuss with the principal investigator prior to the procedure.

5.4.3 Formalin-Fixed Tumor Biopsies

1. Label formalin-filled containers according to instructions in section 5.3.1.1.
2. Obtain the designated number of core biopsies (see Section 5.1) using a 16-gauge or 18-gauge core needle, and place one core in each cassette.
3. Snap the cassette lids closed and place cassettes into a formalin-filled pre-labeled container as soon as possible after collection to prevent air drying. Up to two cassettes may be placed in one formalin jar.
4. Secure the container lids and place the containers into the shipping kit according to instructions in section 5.5. Keep tissue in formalin jars at room temperature until shipment to the ETCTN Biorepository.

5.4.4 Blood Collection for Germline Testing

During the screening period, a blood sample will be collected to serve as a germline sample for evaluation during the whole exome sequencing/RNAseq analysis.

5.4.4.1 Collection of Blood in EDTA Tubes

1. Label EDTA tubes according to the instructions in section 5.3.1.2.
2. Collect 10 mL blood in EDTA tube(s) and gently invert tube to mix.
3. Ship on day of collection (whenever possible) according to instructions below.
4. If blood cannot be shipped on the day of collection (*e.g.*, a late scheduled collection), then refrigerate until shipment.

5.5 Shipping Specimens from Clinical Site to the ETCTN Biorepository

Core biopsies that are fixed in formalin and fresh blood should be shipped as one shipment at ambient temperature, whenever possible. The same box sent with kit contents should be used to ship specimens to the ETCTN Biorepository. In winter months, please include extra insulation, such as bubble wrap, inside the shipping container.

For formalin-fixed biopsies, if the corresponding anatomical pathology report is not available at the time of shipment, then the surgical and/or radiology report must be uploaded to the ETCTN specimen tracking system and included in the package, or the specimen will not be processed.

5.5.1 Specimen Shipping Instructions

Tissue in formalin must be shipped on the day of collection. Collect and ship on Monday through Wednesday.

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

5.5.1.1 Shipping Ambient Tissue and Blood in a Single-Chamber Kit

1. Before packaging specimens, verify that each specimen is labeled according to the instructions above and that the lids of all primary receptacles containing liquid are tightly sealed. Formalin jars should be wrapped in parafilm.
2. Place the specimens in zip-lock bags. Use a separate bag for each specimen type.
3. Place specimens into a biohazard envelope with absorbent material. Expel as much air as possible and seal the envelope securely.
4. Place the biohazard envelope into a Tyvek envelope. Expel as much air as possible and seal securely.
5. Place the specimen(s) and a copy of the shipping manifest and corresponding reports such as surgical or radiology reports into the insulated shipping container. In winter months, please include extra insulation, such as bubble wrap, inside the shipping container, to prevent specimens from freezing.
6. Place the lid on top of the container. Close the outer flaps and tape shut.
7. Attach a shipping label to the top of the shipping container.
8. Attach an Exempt Human Specimen sticker to the side of the container.
9. Ship specimens via overnight courier to the address below. FedEx Priority Overnight is strongly recommended to prevent delays in package receipt.

5.5.2 Shipping Address

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

ETCTN Biorepository
The Research Institute at Nationwide Children's Hospital
700 Children's Drive, WA1340
Columbus, Ohio 43205
PH: (614) 722-2865
FAX: (614) 722-2897
Email: BPCBank@nationwidechildrens.org

FedEx Priority Overnight service is very strongly preferred.

NOTE: The ETCTN Biorepository FedEx Account will not be provided to submitting institutions.

5.5.3 Contact Information for Assistance

For all queries, please use the contact information below:

ETCTN Biorepository
Toll-free Phone: (800) 347-2486
E-mail: BPCBank@nationwidechildrens.org

5.6 Biomarker Plan

List of Biomarker Assays in Order of Priority

Priority	Biomarker Name	Biomarker Assay	Biomarker Type and Purpose	M/O	Timing	Specimen and Quantity Needed	Laboratory
1	Genomic alterations in HR pathway genes	Whole exome sequencing and RNAseq	Integrated To assess for genomic alterations in genes relevant to homologous recombination repair and assess effect of such alterations on clinical response to treatment.	M M	Pre-treatment Pre-treatment	DNA and RNA from Tumor biopsy 2 cores DNA from Whole Blood (for germline) 10 mL	Molecular Characterization and Clinical Assay Development Laboratory (MoCha) Frederick National Laboratory for Cancer Research (NCI) Biswajit Das, PhD
2	Functional HR assay (RAD51)	RAD51 foci, H2AX and geminin by IHC	Integrated To evaluate whether RAD51 foci formation as assessed at baseline or while on-treatment is correlated with response to treatment. To evaluate whether patients who show initial clinical benefit but develop radiographic progressive disease have a change in assay result using an optional biopsy.	M M O	Pre-treatment On-treatment (C2D3-5) Pre-progression	10 unstained slides from tumor biopsy per timepoint	Brigham and Women's Hospital Pathology Core Dr. Geoffrey Shapiro, Dana Farber Cancer Institute
3	SLFN11 expression	SLFN11 expression by IHC and possible mRNA	Exploratory To evaluate whether SLFN11 expression is predictive of clinical response to treatment.	O O	Pre-treatment Pre-progression	10 unstained slides from tumor biopsy per timepoint	Developmental Therapeutics NCI Yves Pommier, MD, PhD
4	MGMT expression	MGMT expression by IHC and possible mRNA	Exploratory To evaluate whether MGMT expression is predictive of clinical response to treatment.	O O	Pre-treatment Pre-progression	10 unstained slides from tumor biopsy per timepoint	Developmental Therapeutics NCI Yves Pommier, MD, PhD

IHC=Immunohistochemistry, NGS=Next generation sequencing, MGMT=O[6]-methylguanine-DNA methyltransferase, SLFN11=Schlafen family member number 11, RAD51= DNA repair protein RAD51 homolog; mRNA = messenger RNA.

5.7 Integrated Correlative Studies

Specimens from this study will be received, accessioned, paraffin embedded and stored per ETCTN Biorepository guidelines. Once the study is completed and all material has been received, nucleic acids/DNA will be isolated per established procedures and sent to the NCLN Genomics laboratory for further analysis. Slides will be made and distributed in batch to the laboratory performing the RAD51 correlative study. The prioritization of tissue for the study is shown in Section 5.6.

5.7.1 Genomic Alterations in HR Pathway Genes

5.7.1.1 Specimen Receipt and Processing at the ETCTN Biorepository

Formalin-fixed tissue from the pre-treatment time point will be used for this assay. Tissue in formalin will be processed and embedded upon receipt at the ETCTN Biorepository, and slides will be cut from the biopsies. For all tumor specimens, the first section will be stained with hematoxylin and eosin (H&E) for pathology quality control review to assess tumor content; unstained slides will be macrodissected, if needed, and scraped for DNA and RNA co-extraction. DNA will be banked in a stock vial and RNA will be divided into 5 aliquots; all nucleic acids will be stored in a -80°C freezer until distribution for testing.

DNA will be extracted from blood collected in an EDTA tube at the pre-treatment time point. DNA will be stored in a -80°C freezer until distribution for testing.

5.7.1.2 Site Performing Correlative Study

Whole exome sequencing (WES) and RNAseq will be conducted in pre-treatment biopsies at the Molecular Characterization and Clinical Development Laboratory (MoCha) of the NCI. The assay will specifically evaluate for genomic mutations, copy number changes and loss of heterozygosity in pre-specified HR pathway genes (BRCA1, BRCA2, ATM, ATR, ATRX, CHK1, CHK2, RAD51, PALB2, FANCA, and NBS1) using the MoCha pipeline. Alterations in other genes will analyzed by the MoCha WES/RNAseq pipeline will also be characterized and reported.

5.7.1.3 Submission of Sequencing Results Previously Obtained as Standard of Care

WES results are available for 16 of the 22 patients who participated on 10250 using tumor biopsies obtained for the study. No results are available for the remaining patients because tissue was not available for various reasons, including technical issues accessing the tumor during the biopsy or poor tumor content. To better understand whether patients with genomic alterations in the HR pathway are more likely to derive clinical benefit from the study treatment and to perform the most complete analysis feasible, we would like to request that sites submit next generation sequencing (NGS) results from NGS assays previously performed as standard of care (i.e. Foundation Medicine, Caris, institutional platform). These reports would be redacted per institutional practice and submitted to the study PI.

5.7.2 RAD51 Foci Formation Assay (Functional HR assay)

5.7.2.1 Specimen Receipt and Processing at the ETCTN Biorepository

For this correlative, 10 unstained , uncharged 4 micron slides from each biopsy (pre-treatment, on-treatment and pre-progression, if applicable) will be created, labeled appropriately, and sent to the laboratory of Dr. Geoffrey Shapiro of Dana Farber Cancer Institute. The slides will be packed at ambient conditions and sent by overnight courier to:

Bose Kochupurakkal, Ph.D.; Re: NCI 10250
Harvard Institutes of Medicine HIM324/331
Center for DNA Damage and Repair
4 Blackfan Circle, Boston, MA 02115

Prior to shipment, an e-mail will be sent to Bose Kochupurakkal (bose_kochupurakkal@dfci.harvard.edu) to inform the laboratory of the shipment.

5.7.2.2 Site Performing Correlative Study

The RAD51 foci formation assay will be performed in pre-treatment, on-treatment and optional 'pre-progression' biopsies at the Brigham and Women's Hospital Pathology Core under the direction of Dr. Geoffrey Shapiro of Dana Farber Cancer Institute. The RAD51 stain will be performed on the VENTANA platform and Geminin stains are performed on the BOND platform using standardized protocols. Antibodies to RAD51, geminin and H2AX are obtained from commercial sources that meet GMP standards. Paraffin sections of a mini-tissue microarray containing irradiated and unirradiated breast cancer cell lines MDA-MB436 (BRCA1 mutant), MDA-MB-468 (BRCA1/2-WT) and the ovarian cancer cell line KURAMOCHI (BRCA2 mutant) will be used as controls.

5.8 Exploratory/Ancillary Correlative Studies

Specimens from this study will be received, accessioned, paraffin embedded and stored per ETCTN Biorepository guidelines. Once the study is completed and all material has been received, slides will be made and distributed in batch to the laboratories performing the MGMT and SLFN11 correlative studies. The prioritization of tissue for the study is shown in Section 5.6.

5.8.1 MGMT Expression

5.8.1.1 Specimen Receipt and Processing at the ETCTN Biorepository

For this correlative, 10 unstained, uncharged slides from the pre-treatment and pre-progression biopsy (if applicable) will be created, labeled appropriately, and sent to the laboratory of Dr. Yves Pommier of the National Cancer Institute for MGMT expression analysis. The slides will be packed at ambient conditions and sent by overnight courier to:

Translational Pharmacodynamics Research Group
Developmental Therapeutics Branch, CCR, NCI, NIH
10 Center Drive
Building 10, Room 12C208
Bethesda, MD 20892
Phone: 240-760-6330

Prior to shipment, an e-mail will be sent to: Jane Trepel (trepelj@mail.nih.gov) and Sunmin Lee (leesun@mail.nih.gov) to inform the laboratory of the shipment.

5.8.1.2 Site Performing Correlative Study

MGMT protein (and possibly RNA expression) will be evaluated using established methodology in pre-treatment and optional 'pre-progression' biopsies in the laboratory of Dr. Yves Pommier, Developmental Therapeutics Branch, NCI.

5.8.2 SLFN11 Expression

5.8.2.1 Specimen Receipt and Processing at the ETCTN Biorepository

For this correlative, 10 unstained, uncharged slides from the pre-treatment and pre-progression biopsy (if applicable) will be created, labeled appropriately, and sent to the laboratory of Dr. Yves Pommier of the National Cancer Institute for SLFN11 expression analysis. The slides will be packed at ambient conditions and sent by overnight courier to:

Translational Pharmacodynamics Research Group
Developmental Therapeutics Branch, CCR, NCI, NIH
10 Center Drive
Building 10, Room 12C208
Bethesda, MD 20892
Phone: 240-760-6330

Prior to shipment, an e-mail will be sent to: Jane Trepel (trepelj@mail.nih.gov) and Sunmin Lee (leesun@mail.nih.gov) to inform the laboratory of the shipment.

5.8.2.2 Site Performing Correlative Study

SLFN11 protein (and RNA expression) will be evaluated using established methodology in pre-treatment and optional 'pre-progression' biopsies by immunohistochemistry and mRNA in the laboratory of Dr. Yves Pommier, Developmental Therapeutics Branch, NCI.

6. TREATMENT PLAN

6.1 Agent Administration

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in Section 10. Appropriate dose modifications are described in Section 7. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy.

Regimen Description					
Agent	***Premedications; Precautions***	***Dose***	***Route***	***Schedule***	***Cycle Length***
Olaparib	Do not consume grapefruit juice	200 mg	Tablets PO BID	Days 1-7	21 days (3 weeks)
Temozolomide (TMZ)	Ondansetron 8 mg PO once with TMZ. Take on an empty stomach	75 mg/m^2	Capsules PO once daily	Days 1-7	

TMZ=temozolomide; PO=oral administration, BID=twice per day.

The patient will be requested to maintain a medication diary of each dose of medication. The medication diary will be returned to clinic staff at the end of each course.

6.1.1 Olaparib

Patients will be administered olaparib twice daily (BID). Olaparib tablets should be taken at the same time each day, approximately 12 hours apart with one glass of water. The first olaparib dose of the day should be taken concomitantly with TMZ. The olaparib tablets should be swallowed whole and not chewed, crushed, dissolved or divided. Olaparib tablets can be taken with or without food; however, since the morning dose is taken with TMZ, both agents will be taken together on an empty stomach for the morning dose. It is prohibited to consume grapefruit, grapefruit juice, or Seville oranges while on olaparib therapy.

If vomiting occurs shortly after the olaparib tablets are swallowed, the dose should only be replaced if all of the intact tablets can be seen and counted. Should any patient enrolled on the study miss a scheduled dose of olaparib for whatever reason (*e.g.*, as a result of forgetting to take the tablets or vomiting), the patient will be allowed to take the scheduled dose of olaparib up to a maximum of 2 hours after that scheduled dose time. If greater than 2 hours after the scheduled dose time, the missed dose of olaparib is not to be taken and the patient should take their allotted dose at the next scheduled time.

6.1.2 Temozolomide

Patients will be administered TMZ once daily (QD). The TMZ capsules should be swallowed whole with a glass of water and not opened or chewed. To reduce nausea, TMZ should be taken

on an empty stomach and patients will take ondansetron 8 mg PO once concurrently with the TMZ dose during cycle 1. If patients have a medical contraindication to ondansetron, another anti-emetic may be substituted. The use of ondansetron beyond cycle 1 is at the discretion of the principle investigator.

TMZ is rapidly and completely absorbed after oral administration with a peak plasma concentration achieved in a median of 1 hour. Food reduces the extent of TMZ absorption.

If vomiting occurs shortly after the temozolomide tablets are swallowed, the dose should only be replaced if all of the intact tablets can be seen and counted. Should any patient enrolled on the study miss a scheduled dose of temozolomide for whatever reason (*e.g.*, as a result of forgetting to take the tablets or vomiting), the patient will be allowed to take the scheduled dose of temozolomide up to a maximum of 12 hours after that scheduled dose time. If greater than 12 hours after the scheduled dose time, the missed dose of temozolomide is not to be taken and the patient should take their allotted dose at the next scheduled time.

6.2 General Concomitant Medication and Supportive Care Guidelines

Because there is a potential for interaction of olaparib with other concomitantly administered drugs, the case report form must capture the concurrent use of all other drugs, over-the-counter medications, vitamins, nutritional supplements, or alternative therapies at the time of enrollment and throughout the study. The case report form should capture the dates of administration (including start/end dates if known), dosage (including dosing frequency/schedule), and reason for use. The Principal Investigator should be alerted if the patient is taking any agent known to affect or with the potential for drug interactions. The study team should check a frequently-updated medical reference for a list of drugs to avoid or minimize use of. Appendix B (Patient Drug Information Handout and Wallet Card) should be provided to patients if available.

6.2.1 Dietary Restrictions and Over-the-Counter/Self-Medication

It is prohibited to consume grapefruit, grapefruit juice, Seville oranges, or Seville orange juice while on olaparib therapy. The use of any natural/herbal products or other traditional remedies should be discouraged.

6.2.2 Medications that May NOT be Administered

No other anti-cancer therapy (chemotherapy, immunotherapy, hormonal therapy [hormone replacement therapy is acceptable], radiotherapy, biological therapy, or other novel agent) is to be permitted while the patient is receiving study medication.

Live virus and live bacterial vaccines should not be administered while the patient is receiving study medication and during the 30-day follow-up period. An increased risk of infection by the administration of live virus and bacterial vaccines has been observed with conventional chemotherapy drugs and the effects with olaparib are unknown.

6.2.3 Restricted Concomitant Medications

6.2.3.1 Strong or Moderate CYP3A Inhibitors

Known strong CYP3A inhibitors (*e.g.*, itraconazole, telithromycin, clarithromycin, boosted protease inhibitors, indinavir, saquinavir, nelfinavir, boceprevir, telaprevir) or moderate CYP3A inhibitors (ciprofloxacin, erythromycin, diltiazem, fluconazole, verapamil) should not be taken with olaparib.

6.2.3.2 Strong or Moderate CYP3A Inducers

Strong (*e.g.*, phenobarbital, phenytoin, rifampicin, rifabutin, rifapentine, carbamazepine, nevirapine, enzalutamide and St John's Wort) and moderate CYP3A inducers (*e.g.*, bosentan, efavirenz, modafinil) of CYP3A should not be taken with olaparib.

6.2.3.3 P-gp inhibitors

It is possible that co-administration of P-gp inhibitors (*e.g.*, amiodarone, azithromycin) may increase exposure to olaparib. Caution should therefore be exercised.

6.2.3.4 Effect of olaparib on other drugs

The efficacy of hormonal contraceptives may be reduced if co-administered with olaparib.

Based on limited *in vitro* data, olaparib may increase the exposure to substrates of CYP3A4, OATP1B1, OCT1, OCT2, OAT3, MATE1, and MATE2K.

Based on limited *in vitro* data, olaparib may reduce the exposure to substrates of CYP2B6.

Caution should therefore be exercised if substrates of these isoenzymes or transporter proteins are co-administered.

Examples of substrates include (use a frequently-updated resource for comprehensive list):

- CYP3A4 – hormonal contraceptive, simvastatin, cisapride, cyclosporine, ergot alkaloids, fentanyl, pimozide, sirolimus, tacrolimus, and quetiapine
- CYP2B6 – bupropion, efavirenz
- OATP1B1 – bosentan, glibenclamide, repaglinide, statins, and valsartan
- OCT1, MATE1, MATE2K – metformin
- OCT2 – serum creatinine
- OAT3 – furosemide, methotrexate

6.2.4 Anticoagulant Therapy

Patients who are taking warfarin may participate in this trial; however, it is recommended that the international normalized ratio (INR) be monitored carefully at least once per week for the first month, then monthly if the INR is stable. Subcutaneous heparin and low molecular weight heparin are permitted.

6.2.5 Anti-emetics/Anti-diarrheals

If a patient develops nausea, vomiting, and/or diarrhea, then these symptoms should be reported as AEs and appropriate treatment of the event given. An information sheet for patients regarding management and reporting of diarrhea can be found in Appendix C.

6.2.6 Administration of other Anti-Cancer Agents

Patients must not receive any other concurrent anti-cancer therapy, including investigational agents, while on study treatment. Patients may continue the use of bisphosphonates or denosumab for bone disease and corticosteroids for the symptomatic control of brain metastases provided the dose is stable before and during the study and the agents were started at least 4 weeks prior to beginning study treatment.

6.3 Duration of Therapy

Treatment will continue until one of the following criteria applies:

- Disease progression per RECIST version 1.1 criteria
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Bone marrow findings consistent with MDS or AML
- Patient decides to withdraw from the study
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- Clinical progression
- Patient non-compliance
- Pregnancy
    - All women of child bearing potential should be instructed to contact the investigator immediately if they suspect they might be pregnant (*e.g.*, missed or late menstrual period) at any time during study participation.

  - The investigator must immediately notify CTEP in the event of a confirmed pregnancy in a patient participating in the study.

- Termination of the study by sponsor

- The drug manufacturer can no longer provide the study agent

The reason(s) for protocol therapy discontinuation, the reason(s) for study removal, and the corresponding dates must be documented in the case report form (CRF).

6.4 Duration of Follow Up

Patients removed from study for adverse events will be followed until resolution or stabilization of the adverse event. Patients will be followed in clinic or by telephone on an approximately every 3 month schedule for 2 years after study participation ends to collect information related to disease status, subsequent anti-cancer therapy and any new diagnosis of AML or MDS. Patients will continue to be followed every 6 months thereafter until death or withdrawal of consent to ascertain any new diagnosis of AML or MDS, and the status of those conditions if diagnosed.

7. DOSE DELAYS AND MODIFICATIONS

7.1 Required Parameters for Initiation of a Treatment Cycle

A new cycle of therapy may begin only if the following criteria are met:

- ANC ≥ 1,000/μL
- Hemoglobin ≥ 9 g/dL (for cycle 1, day 1; ≥ 8 g/dL for all other cycles)
- Platelets ≥ 100,000/μL
- Grade ≥ 2 non-hematologic adverse events (except for alopecia) have resolved to grade ≤ 1, acceptable grade 2 (in the opinion of the treating investigator), or baseline.

7.2 General Principles

The following guidelines apply for dose reductions and delays:

- Dosing for a given cycle will be based on adverse events observed during the prior cycle. When multiple adverse events occur, the modification that would require the patient to receive the lowest dose combination of the study drugs is used.
- When toxicity requires a new treatment cycle to be delayed, both agents should be held until the toxicity resolves and then resumed simultaneously. Deviations from this approach should be discussed with the principal investigator.
- If toxicity requires a study agent to be held during the dosing phase (days 1-7) of a cycle, the other study agent may be continued at the investigator's discretion. If toxicity resolves, treatment with the agent that was held should generally resume at the time of the next scheduled cycle (i.e. the missed doses should not be made up). When an adverse

event results in a study agent being interrupted during a cycle, all planned assessments for that cycle will otherwise occur as scheduled.

- A subject may incur up to 2 dose reductions of each study agent prior to discontinuation of that agent. Furthermore, TMZ may not be dose reduced below 25 mg/m^2 daily, and olaparib may not be dose reduced below 100 mg BID; in the event reduction below such dose level is indicated, that study agent should be discontinued. However, after discontinuation of one study agent, the other study agent may be continued at the discretion of the treating investigator after an assessment of the overall risk-/benefit ratio for the patient.
- For non-hematologic adverse events that have not resolved at the scheduled start of a cycle per Section 7.1, treatment may be delayed for up to 3 weeks. If, after a 3 week delay, adverse events have still not resolved, in general the patient should be removed from the study. For the management of non-resolving hematologic toxicity, please see Section 7.4.3.

7.3 Dose Levels for Olaparib and Temozolomide

The following dose levels for both agents will be used during this study and apply to the recommended management given throughout this section. If a patient requires more than 2 dose reductions of a study agent, that study agent should be discontinued.

Olaparib dose reductions on study treatment

Initial Olaparib Dose	Dose reduction 1	Dose reduction 2
200 mg BID	150 mg BID	100 mg BID

TMZ dose reductions on study treatment

Initial TMZ Dose	Dose reduction 1	Dose reduction 2
75 mg/m^2 QD	50 mg/m^2 QD	25 mg/m^2 QD

7.4 Management of Hematologic Toxicity

7.4.1 General Approach

Observed Hematologic Toxicity	Recommended Management
ANC < 500/μL and/or Platelets < 50,000/μL Grade 3 or 4 neutropenic fever requiring oral or intravenous antibiotics	Delay cycle. Monitor complete blood count at least weekly. When criteria for new cycle are met, resume at one dose level lower for the agent to which toxicity is assessed.

Grade 3 thrombocytopenia with bleeding Hemoglobin < 7 g/dL Other hematologic adverse events ≥ grade 3 deemed clinically significant by the treating investigator.	
ANC 500-999/µL and/or Platelets 50,000/mcL - 99,999/mcL and/or Hemoglobin < 8 g/dL	Delay cycle. Monitor complete blood count at least weekly. When criteria for new cycle are met, resume treatment at same dose level.

7.4.2 Use of transfusions and colony stimulating factors

The use of blood product transfusions (packed red blood cells and platelets) is permitted per institutional guidelines. Erythropoietin stimulating agents may be used per institutional and National Comprehensive Cancer Network (NCCN) guidelines at the treating investigator's discretion. In the event of cytopenias requiring dose modifications and/or delays, the use of G-CSF as primary prophylaxis in subsequent cycles is permitted per institutional guidelines and investigator judgement, particularly in situations where patients appear to be deriving benefit from the study treatment. The use of thrombopoietin agonists for the management of thrombocytopenia is not recommended.

7.4.3 Management of prolonged hematologic toxicity

Should any subject develop evidence of prolonged hematologic toxicity, including but not limited to ≥2 week interruption/delay in study drug administration due to any of the following, additional monitoring is indicated:

- grade 3 anemia (Hgb <8 g/dL)
- grade 3 neutropenia (ANC <1000/mcL)
- grade 3 thrombocytopenia (platelets <50/mcL) and/or
- development of transfusion dependence for red blood cells or platelets

In any of these cases, weekly blood counts including reticulocytes and peripheral smear should be monitored. If toxicity has not resolved to ≤ grade 1 within 3 weeks of study drug treatment being held, the patient should be referred to a hematologist for further evaluation and the patient should be removed from the study. Bone marrow biopsy should be considered for evaluation including cytogenetics. Patients who develop MDS or AML while on study treatment should discontinue study participation and be managed appropriately by a hematologist oncologist.

7.4.4 Dose re-escalation of olaparib

Patients who have undergone dose modification of olaparib may be considered for dose re-escalation of that drug if they complete 2 cycles of treatment without dose delays and/or

modifications (of any study agent). In that situation, the dose of olaparib may only be re-escalated by one dose level on one occasion

7.5 Management of Non-Hematologic Toxicities Attributed to Olaparib

Olaparib may be modified for non-hematologic toxicities per below:

Diarrhea Attributed to Olaparib	Management Guidelines
≤ Grade 1	No change in dose.
Grade 2	No change in dose. Evaluate for infectious etiology if clinically indicated. Institute anti-diarrheal therapy. If diarrhea persists at grade 2 or higher for more than 5 days despite supportive care, interrupt olaparib or delay cycle (as appropriate) and manage supportively. Reinstitute at same dose when symptoms improve to ≤ grade 1. Recurrent grade 2 diarrhea: Interrupt olaparib or delay cycle (as appropriate). Evaluate for infectious etiology. Manage supportively. Upon resolution to ≤ grade 1, resume at one dose level lower for olaparib.
Grade 3	Interrupt olaparib or delay cycle (as appropriate) until ≤ grade 1. Evaluate for infectious etiology if clinically indicated. Institute anti-diarrheal therapy. Resume at one dose level lower for olaparib when symptoms improve to ≤ grade 1.
Grade 4	Discontinue olaparib permanently.

Other Non-Hematologic Toxicity Attributed to Olaparib	Dose Reduction
Grade 1 or 2	No change in dose. Continue therapy at the investigator's discretion.
Grade 3 non-hematologic toxicity deemed clinically significant by the treating investigator and not attributable to TMZ or underlying disease	Interrupt olaparib or delay cycle (as appropriate) until event resolved to grade ≤ 1, acceptable grade 2 (in the opinion of the treating investigator), or baseline. Resume at one dose level lower for olaparib.
Grade 4 non-hematologic toxicity not attributable to TMZ or underlying disease	Discontinue olaparib permanently.

7.5.1 Renal Impairment

Olaparib has not been studied in patients with severe renal impairment (CrCl ≤ 30 mL/min) or end-stage renal disease; if patients develop severe renal impairment (CrCl < 30 mL/min) or end stage renal disease, the case should be discussed with the CTEP Medical Monitor. Investigators

should be aware that olaparib may cause artificial increases in the blood creatinine level. These effects are often mild and occur early in treatment.

7.6 Management of Non-Hematologic Toxicities Related to Temozolomide

Non-Hematologic Toxicity Attributed to TMZ	Dose Reduction
Intolerable grade 2 toxicity	Interrupt TMZ or delay cycle (as appropriate) until resolved to grade 1, or deemed tolerable with supportive care.
Grade 3/4 fatigue, nausea, constipation and/or diarrhea which persist ≥ 3 days despite optimal supportive care Other grade 3 adverse event deemed clinically significant by the treating investigator.	Interrupt TMZ or delay cycle (as appropriate) until event resolved to grade 1, acceptable grade 2 (in the opinion of the treating investigator), or baseline. Resume at one lower dose level for TMZ.
Grade 4 (except for grade 4 alopecia, fatigue, nausea and vomiting not maximally managed with supportive care)	Discontinue TMZ permanently.

7.7 Management of New or Worsening Pulmonary Symptoms

If new or worsening pulmonary symptoms (*e.g.*, dyspnea) or radiological abnormalities occur in the absence of a clear diagnosis, an interruption in study treatment dosing is recommended and further diagnostic workup (including a high-resolution CT scan) should be performed to exclude pneumonitis.

Following investigation, if no evidence of abnormality is observed on CT imaging and symptoms resolve, then study treatment can be restarted, if deemed appropriate by the investigator. If significant pulmonary abnormalities are identified, these need to be discussed with the CTEP Medical Monitor.

7.8 Management of Nausea and Vomiting

Events of nausea and vomiting are known to be associated with olaparib and TMZ treatment. Patients should take ondansetron 8 mg PO once with the AM dose of TMZ for at least 1 cycle. Another anti-emetic can be substituted if clinically indicated.

These events are generally mild to moderate (CTCAE grade 1 or 2) severity, intermittent, and manageable on continued treatment. The first onset generally occurs in the first month of treatment for nausea and within the first 6 months of treatment for vomiting. For nausea, the incidence generally plateaus at around 9 months, and for vomiting at around 6 to 7 months.

Patients should receive additional appropriate anti-emetic treatment at the first onset of nausea or vomiting and as required thereafter, in accordance with local treatment practice guidelines.

As per international guidance on anti-emetic use in cancer patients (ESMO, NCCN), generally a single agent antiemetic should be considered, such as a dopamine receptor antagonist, antihistamine, or dexamethasone.

7.9 Interruptions for Intercurrent Non-Toxicity Related Events

Study treatment dose interruption for conditions other than toxicity resolution should be kept as short as possible. If a patient cannot restart study treatment within 4 weeks for resolution of intercurrent conditions not related to disease progression or toxicity, the case should be discussed with the CTEP Medical Monitor. Patients who are discontinued from the study for reasons other than progression of disease or treatment-related toxicity may be replaced.

All dose reductions and interruptions (including any missed doses), and the reasons for the reductions/interruptions are to be recorded in the eCRF.

Surgery indicated for the management of non cancer-related conditions (e.g. cholecystectomy, appendectomy) is permitted. Study treatment should be stopped at least 7 days prior to planned surgery. After surgery, study treatment can be restarted when the wound has healed. Surgery directed at sites involved by malignancy will usually require cessation of study participation for reasons of disease progression. Surgery that is related to side effects or toxicity from the drug should also result in cessation of study participation. Individual cases may be discussed with the CTEP Medical Monitor. No interruption of study treatment is required for any needle biopsy procedure however a blood count should be checked to ensure counts are adequate.

Because the AEs related to olaparib and TMZ may include asthenia, fatigue, and dizziness, patients should be advised to use caution while driving or using machinery if these symptoms occur.

8. PHARMACEUTICAL INFORMATION

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

8.1 CTEP IND Agent

8.1.1 Olaparib (AZD2281) (NSC 747856)

Chemical Name: 4-[(3-{[4-(cyclopropylcarbonyl)piperazin-1-yl]carbonyl}-4-fluorophenyl)methyl]phthalazin-1(2*H*)-one

Other Names: AZD2281; KU-0059436; CO-CE 42

Classification: PARP inhibitor

CAS Registry Number: 763113-22-0

Molecular Formula: $C_{24}H_{23}FN_4O_3$ **M.W.:** 434.46

Approximate Solubility: 0.1 mg/mL pH independent solubility across physiologic range

Mode of Action: Olaparib is an inhibitor of subclasses 1, 2, and 3 of polyadenosine 5′ diphosphoribose polymerase (PARP-1, PARP-2, and PARP-3). In tumors that are deficient in the homologous recombination DNA repair pathway (example, BRCA mutants), inhibition of PARP by olaparib causes accumulation of DNA double-strand breaks and genomic instability. Olaparib may also enhance the effects of DNA damage caused by ionizing radiation and chemotherapy.

Description: crystalline solid

How Supplied: AstraZeneca supplies and the Pharmaceutical Management Branch (PMB), CTEP, Division of Cancer Treatment and Diagnosis (DCTD) distributes olaparib as green, film-coated tablets in 100 mg and 150 mg strengths.

- 100 mg tablets are 14.5 mm x 7.25 mm oval-shaped
- 150 mg are 14.5 mm x 7.25 mm oval-shaped

Tablets are packaged in induction-sealed high-density polyethylene (HDPE) bottles with child-resistant closures. Each bottle contains 32 tablets with desiccant.

Tablet core components include active drug substance, copovidone, colloidal silicon dioxide, mannitol and sodium stearyl fumarate. Film coating contains hydroxypropyl methylcellulose (hypromellose), macrogol 400 (polyethylene glycol 400), titanium dioxide, iron oxide yellow and iron oxide black.

Storage: Store in a secure location below 30° C (86° F).

If a storage temperature excursion is identified, promptly return olaparib (AZD2281) to room temperature and quarantine the supplies. Provide a detailed report of the excursion (including documentation of temperature monitoring and duration of the excursion) to PMBAfterHours@mail.nih.gov for determination of suitability.

Stability: Shelf-life studies are ongoing. Sites are not permitted to re-package tablets. Once the bottle is opened, olaparib tablets must be used within 3 months of the opening date; unused tablets should be discarded. Instruct patients not to open a bottle until they are ready to use it.

Route and Method of Administration: Oral. Take tablets without regard to meals.

Potential Drug Interactions: *In vivo* data indicate that CYP3A4/5 is important for olaparib metabolism and clearance in humans. For this reason, avoid concomitant administration of

strong and moderate CYP 3A4/5 inducers and inhibitors. Consult the protocol document or study investigator prior to making any dose adjustments related to potential drug-drug interactions.

In vitro data shows olaparib is a substrate for P-glycoprotein (P-gp), but not for organic anion-transporting polypeptides (OATP1B1 and OATP1B3), organic cation transporter 1 (OCT1), multi-drug resistance protein 2 (MRP-2) efflux transporter or breast cancer resistance protein (BCRP). Administration of strong P-gp inhibitors and inducers should be avoided with concurrent olaparib.

Based on *in vitro* data, olaparib inhibits CYP 3A4 and UGT1A1 enzyme systems and induces CYP 1A2, 2B6, and 3A4. Therefore, avoid concomitant administration of sensitive substrates, particularly those with narrow therapeutic ranges.

Olaparib is also an inhibitor of P-gp, OATP1B1, OCT1, OCT2, OAT3, multi-drug and toxin extrusion proteins (MATE1 and MATE2K) and a weak inhibitor of BRCP, but not an inhibitor of OATP1B3 or MRP-2. *In vitro* studies suggest that olaparib may increase exposure of substrates of these transport systems, although the clinical relevance is not clear. The manufacturer recommends that statins, in particular, should be administered with caution when given concomitantly with olaparib.

Patient Care Implications: Pre-clinical data indicate that olaparib adversely affects embryofetal survival and development. Therefore, women of child-bearing potential and their partners should agree to use two (2) highly effective forms of contraception starting at signing of the informed consent, throughout study participation and for at least one (1) month after the last dose of olaparib. It is not known whether olaparib is found in seminal fluid, so as a precaution, male study participants must use a condom during treatment and for three (3) months after the last dose and should avoid fathering a child or donating sperm during this same time period. The study investigator should discuss the most appropriate forms of highly effective contraceptive methods for each patient.

Lactation is a protocol exclusion criterion and not advised since there is potential for serious adverse reactions in breastfed infants. Advise lactating women to not breastfeed during study treatment and for one (1) month after receiving the last dose of olaparib.

Because the adverse events related to olaparib may include asthenia, fatigue and dizziness, patients should be advised to use caution while driving or using machinery.

There are no data on the effect of olaparib on wound healing, therefore as a precaution, olaparib treatment should be stopped at least 3 days prior to planned surgery. After surgery olaparib can be restarted when the wound has healed. No stoppage of olaparib is required for any needle biopsy procedure.

Study treatment should be discontinued for a minimum of 3 days before a patient undergoes therapeutic or palliative radiation treatment. Study treatment should be restarted within 4 weeks as long as any bone marrow toxicity has recovered.

Availability

Olaparib (AZD2281) is an investigational agent supplied to investigators by the DCTD, NCI.

Olaparib (AZD2281) is provided to NCI under a Collaborative Agreement between the Pharmaceutical Collaborator and DCTD, NCI (see Section 13.4).

8.1.2 Agent Ordering and Agent Accountability

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 active person 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.

Starter supplies will not be provided. Patients must be registered prior to agent ordering. Sites may request expedited orders Monday-Thursday when they provide courier information.

8.1.2.1 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.

8.1.3 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 active person registration status. Questions about IB access may be directed to the PMB IB Coordinator via email.

8.1.4 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)

8.2 Commercial Agent

8.2.1 Temozolomide (NSC 362856)

Chemical Name: 3,4-dihydro-3-11 methyl-4-oxoimidazo[5,1-d]-*a*s-tetrazine-8-carboxamide

Other Names: Temodar®, Temodal®, Temcad®

Classification: Alkylating Agent

CAS Registry Number: 85622-93-1

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 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.

How Supplied: Temozolomide is commercially available. Please refer to the FDA-approved package insert for this drug.

Capsules are supplied in child-resistant sachets containing the following capsule strengths: 5 mg, 20 mg, 100 mg, 140 mg and180 mg.. The dose of temozolomide is based on body surface area (BSA) and 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

Method of Administration: Take each day's dose of capsules at one time, with a full glass of water. Temozolomide will be administered orally. The capsules should be swallowed whole and never chewed. The incidence of nausea and vomiting is decreased when temozolomide is taken on an empty stomach.

9. STATISTICAL CONSIDERATIONS

9.1 Study Design/Endpoints

9.1.1 Study Design

A single arm, open label design will be used to determine the efficacy of the combination of olaparib and TMZ in patients of advanced uterine LMS.

9.1.2 Primary Endpoint

The primary endpoint of this study is the confirmed ORR (complete response + partial response) as measured by RECIST version 1.1 criteria. Responses must occur within the first 6 months of study treatment for the purpose of evaluating the primary endpoint. The ORR will be reported with a 95% confidence interval.

FDA approved second-line and later line agents used in uterine LMS based on phase 3 clinical trial data include trabectedin (a DNA damaging cytotoxic agent) and pazopanib (an anti-angiogenic receptor tyrosine kinase inhibitor). A recent phase 3 clinical trial randomized patients with LMS and another sarcoma subtype (liposarcoma) to trabectedin or dacarbazine. Dacarbazine, an intravenous chemotherapeutic with an analogous mechanism of action to oral TMZ, has often been used as a control arm in phase 3 sarcoma studies, and was used by the European Organization for Research and Treatment of Cancer to establish benchmarks for assessing the activity of novel second line agents in STS (Van Glabbeke *et al.*, 2002). In the randomized phase 3 study of trabectedin versus dacarbazine, patients were required to have previously received an anthracycline-based regimen and there was no upper limit on the number of prior treatments received. In a recently reported subset analysis of the uterine LMS cohort from this study, the ORR was 11% for trabectedin and 9% for dacarbazine and this difference was not statistically significant (p=0.82) (Demetri *et al.*, 2016; Hensley *et al.*, 2017). Similarly, in an analysis of 318 LMS patients enrolled on a single-arm, open-label trabectedin extended access program available after progression on standard chemotherapy, the response rate was 7.5% (Samuels *et al.*, 2013).

In another phase 3 study patients with several sarcoma subtypes who had received at least one regimen containing anthracycline and a maximum of four previous lines of systemic therapy were randomized to pazopanib versus placebo (van der Graaf *et al*., 2012). In a recently published subset analysis of this study (and the preceding phase 2 study) limited to uterine sarcomas (90% had LMS), the response rate to pazopanib was 10% (Benson *et al.*, 2016).

In view of the above, we will consider a response rate of 10% to be inactive and unworthy of further study, whereas a response rate of 35% would be promising for further study among patients with advanced LMS treated with at least one prior systemic regimen. A response rate of 35% for the TMZ + PARPi combination would also be suggestive of superior efficacy over TMZ monotherapy in sarcoma. As discussed above, dacarbazine, the IV analogue of TMZ, was associated with a response rate of 9% among LMS patients treated on the randomized phase 3 study of trabectedin versus dacarbazine (Demetri *et al.*, 2009). Furthermore, in a phase 3 randomized study of eribulin versus dacarbazine which included 75% LMS patients, the response rate to dacarbazine was 5% (Schoffski *et al*., 2016). In a randomized study of gemcitabine and dacarbazine versus dacarbazine alone, the response rate to dacarbazine was 4% (Garcia-del-Muro *et al.*, 2011).

9.1.3 Analysis Plan

A one-stage binomial design will be used evaluating an objective response rate of at most 10% (null hypothesis) versus at least 35% (alternative hypothesis). The design calls for 22 patients. If 5 or more from the total sample of 22 respond, the treatment is considered worthy of further consideration. This design yields 93% power and 1-sided type I error of 6% to test for a response rate of 10% vs. 35%. The study team believes this design, and an observed response rate of 22.7%, would reflect a clinically meaningful finding worthy of further evaluation considering currently available treatment options and results of other studies as described above. A minimum of 22 to a maximum of 25 (22 + 3) patients will be required for the study, allowing for patients deemed ineligible or withdrawing consent prior to the first dose of study treatment (that is, 2-3 additional patients will be recruited to account for non-evaluable patients).

The proposed endpoints are reflective of recently reported phase 2 studies conducted specifically in LMS. Phase 2 studies of pembrolizumab (George *et al.*, 2017), alisertib (Hyman *et al.*, 2017), ixabepilone (Duska *et al*., 2014) and ziv-aflibercept (Mackay *et al*., 2012) conducted specifically in LMS used Simon 2 stage designs with objective response rate as the primary endpoint and a null hypothesis of 5% as compared an alternative hypothesis of between 20-25%, and none met this endpoint (Ben-Ami *et al.*, 2017; Hyman *et al*., 2017; Duska *et al.*, 2014; Mackay *et al.*, 2012). After review of all recently published LMS-specific studies, the highest benchmarks for activity were used in phase 2 studies of trabectedin and sunitinib (10% considered inactive and 30% active) (Monk *et al*., 2012; Hensley *et al*., 2009).

9.2 Sample Size/Accrual Rate

Using the ECTCN network and recognizing that LMS is a relatively common sarcoma subtype, we expect to enroll approximately 2 patients per month across all participating centers. Thus, we anticipate full accrual after approximately 10 months.

PLANNED ENROLLMENT REPORT

Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/ Alaska Native	0	0	0	0	**0**
Asian	1	0	0	0	**1**
Native Hawaiian or Other Pacific Islander	0	0	0	0	**0**
Black or African American	4	0	0	0	**4**
White	12	0	3	0	**15**
More Than One Race	2	0	0	0	**2**
Total	**19**	**0**	**3**	**0**	**22**

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9.3 Stratification Factors

Not applicable.

9.4 Analysis of Secondary Endpoints

9.4.1 Secondary Endpoints

9.4.1.1 Toxicity

Adverse events will be recorded at each clinical visit and will be categorized according to NCI CTCAE version 5.0. Adverse event rates will be reported as counts and percentages per adverse event by grade.

9.4.1.2 Progression-Free Survival

PFS is defined as the time from first treatment with the study drug to the earliest of either disease progression or death from any cause. Patients who are alive and progression free will be censored at the time of their last tumor assessment. The Kaplan-Meier method will be used to evaluate time to event endpoints. Median PFS will be reported with a 95% confidence interval.

9.4.1.3 Integrated Correlatives

The association between ORR, PFS, and the results of integrated assays evaluating for presence of HR deficiency in the tumor will be evaluated. The two integrated assays are genomics for alterations in HR genes and RAD51 foci formation. Both of these assays report binary results. The rate of response will be compared between binary variables using the Fisher's exact test. The log-rank test will be used to compare PFS between binary variables. Additional results from whole exome sequencing and RNAseq analysis on study samples will be reported in a descriptive fashion.

9.4.1.4 Exploratory Correlatives

The association between ORR, PFS, and the exploratory assays will be evaluated in a preliminary fashion. The exploratory assays include SLFN11 and MGMT expression by IHC. SLFN11 and MGMT mRNA expression may also be performed. SLFN11 and MGMT expression are reported as a continuous variable. Logistic regression will be used to estimate the odds of response for every unit increase in protein expression. Cox-regression will be used to evaluate the association between PFS and protein expression. Graphical displays such as box plots and Kaplan-Meier plots will be used to visualize the data.

9.5 Reporting and Exclusions

9.5.1 Evaluation of Toxicity

All patients will be evaluable for toxicity from the time of their first dose of either study agent.

9.5.2 Evaluation of Response

All patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each patient will be assigned one of the following categories: 1) complete response (CR), 2) partial response (PR), 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data). [Note: By arbitrary convention, category 9 usually designates the "unknown" status of any type of data in a clinical database.]

All patients who met the eligibility criteria (with the possible exception of those who received no study medication) should be included in the main analysis of the response rate. Patients in response categories 4-9 should be considered to have a treatment failure (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate. Precise definitions for categories 4-9 will be protocol specific.

All conclusions should be based on all eligible patients. Sub analyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been

identified (*e.g.*, early death due to other reasons, early discontinuation of treatment, major protocol violations, etc.). However, these sub analyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported. The 95% confidence intervals should also be provided.

10. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

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

10.1 Comprehensive Adverse Events and Potential Risks Lists (CAEPRs)

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. *Frequency is provided based on 3449 patients.* Below is the CAEPR for Olaparib (AZD2281).

> **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.

10.1.1 CAEPRs for Olaparib (AZD2281) (NSC747856)

Version 2.5, July 1, 2021[1]

Adverse Events with Possible Relationship to Olaparib (AZD2281) (CTCAE 5.0 Term) [n= 3449]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
Anemia			***Anemia (Gr 4)***
		Febrile neutropenia	
GASTROINTESTINAL DISORDERS			
	Abdominal distension		
Abdominal pain			***Abdominal pain (Gr 3)***
	Constipation		***Constipation (Gr 2)***
Diarrhea			***Diarrhea (Gr 3)***

Adverse Events with Possible Relationship to Olaparib (AZD2281) (CTCAE 5.0 Term) [n= 3449]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
	Dyspepsia		***Dyspepsia (Gr 2)***
	Mucositis oral		
Nausea			***Nausea (Gr 3)***
Vomiting			***Vomiting (Gr 3)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Edema limbs		
Fatigue			***Fatigue (Gr 3)***
IMMUNE SYSTEM DISORDERS			
		Allergic reaction	
INFECTIONS AND INFESTATIONS			
	Upper respiratory infection		
	Urinary tract infection		
INVESTIGATIONS			
	Creatinine increased		
	Neutrophil count decreased		***Neutrophil count decreased (Gr 4)***
		Platelet count decreased	
	White blood cell decreased		
METABOLISM AND NUTRITION DISORDERS			
Anorexia			***Anorexia (Gr 2)***
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Arthralgia		
	Back pain		***Back pain (Gr 2)***
	Muscle cramp		
	Myalgia		
	Pain in extremity		
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS)			
		Leukemia secondary to oncology chemotherapy	
		Myelodysplastic syndrome	
NERVOUS SYSTEM DISORDERS			
	Dizziness		***Dizziness (Gr 2)***
	Dysgeusia		***Dysgeusia (Gr 2)***
	Headache		***Headache (Gr 2)***
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Cough		***Cough (Gr 2)***
	Dyspnea		***Dyspnea (Gr 2)***
		Pneumonitis	
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Rash maculo-papular		
		Skin and subcutaneous tissue disorders - Other (angioedema)	
		Skin and subcutaneous tissue disorders - Other (erythema nodosum)	

NOTE: New Primary Malignancies other than MDS/AML

New primary malignancies have been reported in <1% of patients. There were other contributing factors/potential alternative explanations for the development of the new primary malignancy in all cases, including documented *BRCA* mutation, treatment with radiotherapy and extensive previous chemotherapy including carboplatin, taxanes, anthracyclines and other alkylating and DNA damaging agents. Most are not attributed to olaparib.

[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 olaparib (AZD2281) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that olaparib (AZD2281) caused the adverse event:

CARDIAC DISORDERS - Atrial fibrillation; Cardiac disorders - Other (nodal rhythm); Chest pain - cardiac; Sinus bradycardia; Sinus tachycardia
EAR AND LABYRINTH DISORDERS - Tinnitus
ENDOCRINE DISORDERS - Hypothyroidism
GASTROINTESTINAL DISORDERS - Ascites; Colitis; Colonic obstruction; Dry mouth; Dysphagia; Enterocolitis; Esophageal stenosis; Flatulence; Gastroesophageal reflux disease; Gastrointestinal disorders - Other (gastrointestinal hemorrhage); Gastrointestinal disorders - Other (intestinal obstruction); Gastrointestinal disorders - Other (intestinal perforation); Ileus; Jejunal perforation; Obstruction gastric; Pancreatitis; Periodontal disease; Rectal hemorrhage; Small intestinal obstruction; Stomach pain
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Death NOS; Fever; Malaise; Non-cardiac chest pain
IMMUNE SYSTEM DISORDERS - Immune system disorders - Other (systemic inflammatory response syndrome)
INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Dermatitis radiation; Fracture; Gastrointestinal anastomotic leak; Injury, poisoning and procedural complications - Other (vena cava injury); Wound dehiscence
INVESTIGATIONS - Alanine aminotransferase increased; Aspartate aminotransferase increased; Blood bilirubin increased; GGT increased; Hemoglobin increased; Lipase increased; Lymphocyte count decreased; Serum amylase increased; Weight loss
METABOLISM AND NUTRITION DISORDERS - Dehydration; Hyperglycemia; Hypermagnesemia; Hypocalcemia; Hypokalemia; Hypomagnesemia; Hyponatremia
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Avascular necrosis; Bone pain; Generalized muscle weakness; Muscle weakness lower limb; Muscle weakness upper limb; Neck pain; Rotator cuff injury; Soft tissue necrosis lower limb
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Treatment related secondary malignancy; Tumor pain
NERVOUS SYSTEM DISORDERS - Amnesia; Ataxia; Cognitive disturbance; Concentration impairment; Encephalopathy; Intracranial hemorrhage; Peripheral sensory neuropathy; Reversible posterior leukoencephalopathy syndrome; Stroke; Syncope; Transient ischemic attacks
PSYCHIATRIC DISORDERS - Anxiety; Confusion; Delirium; Hallucinations; Insomnia
RENAL AND URINARY DISORDERS - Acute kidney injury; Renal and urinary disorders - Other (decreased glomerular filtration rate); Renal and urinary disorders - Other (hydronephrosis); Urinary tract obstruction
REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Vaginal hemorrhage
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Bronchopulmonary hemorrhage; Hypoxia; Oropharyngeal pain; Pleural effusion; Respiratory failure; Respiratory, thoracic and mediastinal disorders - Other (chronic obstructive pulmonary disease)
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Alopecia; Erythema multiforme; Pruritus

VASCULAR DISORDERS - Arterial thromboembolism; Flushing; Hot flashes; Hypertension; Hypotension; Peripheral ischemia; Thromboembolic event

Note: Olaparib (AZD2281) 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.

10.1.2 Adverse Event List for TMZ

Common Adverse Effects

- Dermatologic: Alopecia (55% to 69%)
- Gastrointestinal: Constipation (33%), Nausea and vomiting (42% to 53%)
- Hematologic: Neutropenia (10%), Thrombocytopenia (14%)
- Neurologic: Headache (41%), Seizure (23%)
- **Other:** Fatigue

For complete information on potential AEs for TMZ, please refer to the Temodar® Package insert.

10.2 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 5.0. A copy of the CTCAE version 5.0 can be downloaded from the CTEP web site http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

- **For expedited reporting purposes only:**
  - AEs for the agent that are ***bold and italicized*** in the CAEPR (*i.e.*, those listed in the SPEER column, Section 10.1) should be reported through CTEP-AERS only if the grade is above the grade provided in the SPEER.
  - Other AEs for the protocol that require expedited reporting are outlined in Section 10.3.4.

- **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.

10.3 Expedited Adverse Event Reporting

10.3.1 CTEP-AERS

Expedited AE reporting for this study must use CTEP-AERS (CTEP Adverse Event Reporting System), accessed via the CTEP Web site (https://eapps-ctep.nci.nih.gov/ctepaers). 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/protocolDevelopment/electronic_applications/adverse_events.htm). These requirements are briefly outlined in the tables below (Section 10.3.3).

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.

10.3.2 Distribution of Adverse Event Reports

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

10.3.3 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. Attribution to treatment or other cause must be provided.

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

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

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)
NOTE: Investigators **MUST** immediately report to the sponsor (NCI) **ANY** 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 electronic submission 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 submitted electronically 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 electronically 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

10.3.4 Olaparib Adverse Events of Special Interest

AEs of special interest (AESI) are events of scientific and medical interest specific to the further understanding of olaparib's safety profile and require close monitoring and rapid communication by the investigators to CTEP. An AESI may be serious or non-serious. The AESI for olaparib are the following Important Potential Risks:

- MDS/AML
- New primary malignancy (other than MDS/AML)
- Pneumonitis
- Cases of potential drug-induced liver injury (DILI) that include an elevated ALT or AST in combination with either an elevated bilirubin or clinical jaundice, as defined by Hy's Law (see Appendix D) and based on the following observations:
    - Treatment-emergent ALT or AST >3×ULN (or >3× baseline value in

disease states where LFTs may be elevated at baseline) in combination with total bilirubin >2×ULN (of which ≥35% is direct bilirubin).
- Treatment-emergent ALT or AST >3×ULN (or >3× baseline value in disease states where LFTs may be elevated at baseline) in combination with clinical jaundice.

Any event of MDS/AML, new primary malignancy, pneumonitis, or potential DILI should be reported in an expedited manner to CTEP via CTEP-AERS regardless of whether it is considered a non-serious AE (*e.g.*, non-melanoma skin cancer) or SAE, and regardless of the investigator's assessment of causality or knowledge of the treatment arm. For cases of potential DILI, report all relevant laboratory abnormalities and related AEs using the appropriate CTCAE terms.

Events of MDS, AML, or new primary malignancy occurring more than 30 days after the last study treatment must be reported via CTEP-AERS regardless of seriousness or causality. Investigators will be asked during the regular follow-up for OS if the patient has developed MDS/AML or a new primary malignancy and prompted to report any such cases.

10.4 Routine Adverse Event Reporting

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

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

10.5 Pregnancy

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

10.6 Secondary Malignancy

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

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

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

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

10.7 Second Malignancy

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

10.8 Overdose

There is currently no specific treatment in the event of overdose with olaparib and possible symptoms of overdose are not established. Olaparib and Temozolomide must only be used in accordance with the dosing recommendations in this protocol. Any dose or frequency of dosing that exceeds the dosing regimen specified in this protocol should be reported as an overdose. Adverse reactions associated with overdose should be treated symptomatically and should be managed appropriately.

11. STUDY CALENDAR

	Pre-study[1]	Cycle 1[2]			Cycle 2[2]			Cycle 3+[2]			Pre-POD	Off Study[3]
		Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8	Wk 9		
Olaparib[A]		X[A]			X[A]			X[A]				
TMZ[B]		X[B]			X[B]			X[B]				
Informed consent	X											
Demographics	X											
Medical history	X											
Concurrent meds	X	X			X			X				X
Physical exam[C]	X	X			X			X				X
Vital signs[D]	X	X			X			X				X
Height	X											X
Weight	X	X[K]			X[K]			X[K]				X

	Pre-study[1]	Cycle 1[2]			Cycle 2[2]			Cycle 3+[2]			Pre-POD	Off Study[3]
		Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8	Wk 9		
Performance status	X	X			X			X				X
CBC w/diff[E]	X	X[K]	X[L]	X[L]	X[K]	X[L]	X[L]	X[K]		X[L]		X
Serum chemistry[E]	X	X[K]			X[K]			X[K]				X
Coagulation factors[F]	X	X[K]										X
Pregnancy test[G]	X	X			X			X				X
ECG[H]	X											X
Adverse events	X	X----------X										X
Review pill diary		X			X			X				
Imaging evaluation of disease status[I]	X						X					X
Tumor Biopsy	X[M]				X[M]						X[N]	
Blood collection for germline testing[J]	X											

1: All pre-study screening procedures must be completed within 14 days of C1D1, except: informed consent must be completed within 21 days of C1D1, and baseline radiologic evaluations of disease status must be completed within 21 days of C1D1. Blood testing and imaging obtained as part of standard of care and which falls within this timeframe and otherwise meets protocol specifications need not be repeated.

2: A new cycle may begin ± 2 days from scheduled date.

3: Off-study evaluations must be performed within 21 days of the last dose of study drug.

A: Olaparib: 200 mg PO BID on days 1-7 of a 21-day cycle. Dosing window of ± 2 days at the start of each cycle is allowed; however both agents must begin dosing on the same day.

B: TMZ: 75 mg/m^2 PO daily on days 1-7 of a 21-day cycle. Dose is based on weight at screening. Dosing window of ± 2 days at the start of each cycle is allowed; however both agents must begin dosing on the same day. TMZ dose is rounded to the nearest 5 mg increment.

C: A complete physical exam is performed at screening and end-of-study. A limited, symptom based, physical exam is performed at other timepoints. For patients who remain on study as of 2/2023 and live distantly (more than 50 miles) from the site, in-person clinical visits are required every other cycle. During intervening cycles, a telephone or TeleHealth visit may be conducted instead of an in-person visit. Height, weight and vital signs may be omitted. Laboratory tests, except pregnancy test, must still be performed, and can be performed in a local laboratory, prior to the start of the new cycle.

D: Vital signs includes measurement of temperature, heart rate, blood pressure, respiratory rate, and oxygen saturation (pulse oximetry).

E: CBC with diff must include: hemoglobin, red blood cells [RBC], platelets, mean cell volume [MCV], mean cell hemoglobin concentration [MCHC], mean cell hemoglobin [MCH], white blood cells [WBC], absolute differential white cell count (neutrophils, lymphocytes, monocytes, eosinophils and basophils).
Serum chemistry must include: albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, GGT, glucose, LDH, magnesium, phosphorus, potassium, total protein, SGOT (AST), SGPT([ALT]), sodium.

F: Coagulation factors include: aPTT, INR; required pre-study and as clinically indicated. Patients who are on anti-coagulants should have coagulation studies monitored at more frequent intervals for safety - at least once per week for the first two cycles. Local laboratory testing is acceptable.

G: Serum or urine pregnancy test (for women of childbearing potential) must be completed within 14 days of the start of study treatment, on C1D1 of the study prior to commencing treatment, at each subsequent visit during study treatment, and at the follow up visit. Tests will be performed by the treating institution/hospital's laboratory. If results are positive the patient is ineligible and must be discontinued from study treatment immediately. Details of the pregnancy tests must be recorded in the patient's medical records.

H A 12-lead ECG is performed at screening and end-of-study. Additional ECGs are performed as clinically indicated.

I: MRI or CT imaging to evaluate disease status is performed at baseline and once every 2 cycles (6 weeks) ± 3 days. For patients who continue beyond 12 cycles, MRI or CT imaging may be changed to once every 4 cycles (12 weeks) ± 3 days

J: See section 5.4.5 for details on sample collection.

K: Start- of cycle safety assessments may be performed up to 3 days prior to initiating treatment for a new cycle. Local laboratory testing is acceptable.

L: Between cycle safety assessments may be performed ± 3 days from scheduled date. Local laboratory testing is acceptable. If there is a change in the patient's clinical condition after the laboratory testing is complete but before dosing begins, testing should be repeated.

M:: Pre-study biopsy is performed within 14 days of C1D1. On study biopsy is performed on day 3-5 of Cycle 2. If the start of C2 is delayed, the biopsy should be delayed accordingly to match actual drug administration of C2 D3-5. See Correlative Studies section 5 for additional information.

N: Selected patients undergo an optional third tumor biopsy in the event of radiographic progression after initial evidence of clinical benefit. See Section 5.4.2 for details.

TMZ=temozolomide; PO=oral administration, BID=twice per day, QD=once per day; BUN=blood urea nitrogen; GGT=gamma galactosyltransferase; LDH=lactic dehydrogenase; SGOT(AST)=serum glutamic oxaloacetic transaminase (aspartate transaminase); SGPT(ALT)=serum glutamic pyruvic transaminase (alanine transaminase); aPTT=activated partial thromboplastin time; INR=international normalized ratio; MRI=magnetic resonance imaging; CT=computerized tomography; ECG=electrocardiogram; POD=progression of disease.

All time windows refer to business days (*e.g*. exclude weekends and holidays).

11.1 Laboratory Assessments

Full hematology assessments for safety (hemoglobin, red blood cells [RBC], platelets, mean cell volume [MCV], mean cell hemoglobin concentration [MCHC], mean cell hemoglobin [MCH], white blood cells [WBC], absolute differential white cell count (neutrophils, lymphocytes, monocytes, eosinophils and basophils) and absolute neutrophil count or segmented neutrophil count and band forms should be performed at each visit and when clinically indicated. If absolute differentials are not available, please provide % differentials.

Serum biochemistry assessments for safety include sodium, potassium, calcium, magnesium, fasting glucose, creatinine, total bilirubin, gamma galactosyltransferase (GGT), alkaline phosphatase (ALP), aspartate transaminase (AST), alanine transaminase (ALT), urea or blood urea nitrogen (BUN), total protein, albumin, and lactic dehydrogenase (LDH). These assessments should be performed at every clinic visit and when clinically indicated.

Coagulation (activated partial thromboplastin time [aPTT] and INR) will be performed at baseline and if clinically indicated unless the patient is receiving warfarin. Patients taking warfarin may participate in this study; however, it is recommended that prothrombin time (INR and aPTT) be monitored carefully at least once per week for the first two cycles.

Bone marrow or blood cytogenetic samples may be collected for patients with prolonged hematological toxicities as defined in Section 7.4.3. Bone marrow analysis should include an aspirate for cellular morphology, cytogenetic analysis and flow cytometry, and a core biopsy for bone marrow cellularity. If it is not possible to conduct cytogenetic analysis or flow cytometry on the bone marrow aspirate, then attempts should be made to carry out the tests on a blood sample. Full reports must be provided by the investigator for documentation on the Patient Safety database. These data are not required to be entered into CRF.

Pregnancy tests on blood or urine samples will be performed for women of childbearing potential within 14 days prior to the start of study treatment, on Day 1 of the study prior to commencing treatment, at each subsequent visit during study treatment, and at the follow up visit. Tests will be performed by the hospital's local laboratory. If results are positive the patient is ineligible/must be discontinued from study treatment immediately. Details of the pregnancy tests must be recorded in the patient's medical records.

These tests will be performed by the hospital's local laboratory. Additional analyses may be performed if clinically indicated. Local laboratory testing is acceptable as indicated in the study calendar, except for pregnancy testing, which should be performed by the treating institution/hospital's local laboratory.

Any clinically significant abnormal laboratory values should be repeated as clinically indicated and recorded on the eCRF.

In case a patient shows an AST **or** ALT ≥3×ULN **or** total bilirubin ≥2×ULN, please refer to Appendix F, "Actions Required in Cases of Combined Increase of Aminotransferase and Total Bilirubin – Hy's Law", for further instructions. In addition, evaluate patients for potential drug-induced liver injury (DILI) as described in Section 10.3.4.

11.2 ECG

ECGs are required within 14 days prior to starting study treatment, at the end of study treatment, and when clinically indicated.

Twelve-lead ECGs will be obtained after the patient has been rested in a supine position for at least 5 minutes in each case. The investigator or designated physician will review the paper copies of each of the timed 12-lead ECGs on each of the study days when they are collected. ECGs will be recorded at 25 mm/sec. All ECGs should be assessed by the investigator as to whether they are clinically significantly abnormal / not clinically significantly abnormal. If there is a clinically significant abnormal finding, the Investigator will record it as an AE on the eCRF. The original ECG traces must be stored in the patient medical record as source data.

12. MEASUREMENT OF EFFECT

12.1 Antitumor Effect – Solid Tumors

For the purposes of this study, patients should be re-evaluated for response every 6 weeks. In addition to a baseline scan, confirmatory scans should also be obtained 6 weeks (not less than 4) weeks following initial documentation of objective response. For patients who continue beyond 12 cycles, MRI or CT imaging may be changed to once every 4 cycles (12 weeks) ± 3 days

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) (Eisenhauer *et al.*, 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.

12.1.1 Definitions

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

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

according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of 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.

12.1.2 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 (≥2 cm) by chest x-ray or as ≥10 mm (≥1 cm) with CT scan, MRI, or calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Note: Tumor lesions that are situated in a previously irradiated area are not to be considered measurable.

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm (≥1.5 cm) in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm [0.5 cm]). 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 [<1 cm] or pathological lymph nodes with ≥10 to <15 mm [≥1 to <1.5 cm] 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.

12.1.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed 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. PET imaging is not typically used for the radiographic evaluation of soft tissue sarcoma with the possible exception of gastrointestinal stromal tumors. In addition, olaparib is known to have effects on vascularity which could influence the results of PET and be of uncertain clinical significance. Therefore, the use of PET (even with dedicated CT) imaging is not permitted for patients on this study.

Clinical lesions Clinical lesions will only be considered measurable when they are superficial (*e.g.*, skin nodules and palpable lymph nodes) and ≥10 mm (≥1 cm) 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 (0.5 cm) or less. If CT scans have slice thickness greater than 5 mm (0.5 cm), the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (*e.g.* for body scans).

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

scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

PET-CT The use of PET imaging is not permitted for the evaluation of disease status on this study. If, prior to enrollment, a patient underwent PET with dedicated CT, the dedicated CT component may still be used as the baseline imaging study.

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 (Rustin *et al.*, 20004; Bubley *et al.*, 1999; Scher *et al.*, 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 (Vergote et al., 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 The use of FDG PET imaging is not permitted for the evaluation of disease status on this study.

12.1.4 Response Criteria

12.1.4.1 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 (<1 cm).

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 (0.5 cm). (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.

12.1.4.2 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 [<1 cm] 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).

12.1.4.3 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

12.1.5 Progression-Free Survival

See Section 9.4.1.2.

13. STUDY OVERSIGHT AND DATA REPORTING / REGULATORY REQUIREMENTS

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

13.1 Study Oversight

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

During the Phase 2 portion of the study, the Protocol Principal Investigator will have, at a minimum, quarterly conference calls with the Study Investigators and the CTEP Medical Officer(s) to review accrual, progress, and pharmacovigilance.

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

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

13.2 Data Reporting

Data collection for this study will be done exclusively through Medidata Rave. Access to the trial in Rave is granted through the iMedidata application to all persons with the appropriate roles assigned in the Regulatory Support System (RSS). To access Rave via iMedidata, the site user must have an active CTEP IAM account (check at https://ctepcore.nci.nih.gov/iam) and the appropriate Rave role (Rave CRA, Rave Read-Only, Rave CRA (Lab Admin), Rave SLA, or Rave Investigator) on either the LPO or participating organization roster at the enrolling site. To the hold Rave CRA role or Rave CRA (Lab Admin) role, the user must hold a minimum of an AP registration type. To hold the Rave Investigator role, the individual must be registered as an NPIVR or IVR. Associates can hold read-only roles in Rave.

Upon initial site registration approval for the study in RSS, all persons with Rave roles assigned on the appropriate roster will be sent a study invitation e-mail from iMedidata. To accept the invitation, site users must log into the Select Login (https://login.imedidata.com/selectlogin) using their CTEP-IAM user name and password, and click on the "accept" link in the upper right-corner of the iMedidata page. Please note, site users will not be able to access the study in Rave until all required Medidata and study specific trainings are completed. Trainings will be in the form of electronic learnings (eLearnings), and can be accessed by clicking on the link in the upper right pane of the iMedidata screen.

Users that have not previously activated their iMedidata/Rave account at the time of initial site registration approval for the study in RSS will also receive a separate invitation from iMedidata to activate their account. Account activation instructions are located on the CTSU website, Rave tab under the Rave resource materials (Medidata Account Activation and Study Invitation Acceptance). Additional information on iMedidata/Rave is available on the CTSU members' website under the Rave tab or by contacting the CTSU Help Desk at 1-888-823-5923 or by e-mail at ctsucontact@westat.com.

13.2.1 Method

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

13.2.2 Responsibility for Data Submission

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

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

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

An End of Study CRF is to be completed by the PI, and is to include a summary of study endpoints not otherwise captured in the database, such as (for phase 1 trials) the recommended phase 2 dose (RP2D) and a description of any dose-limiting toxicities (DLTs). CTMS will utilize a core set of eCRFs that are Cancer Data Standards Registry and Repository (caDSR) compliant (http://cbiit.nci.nih.gov/ncip/biomedical-informatics-resources/interoperability-and-semantics/metadata-and-models). Customized eCRFs will be included when appropriate to meet unique study requirements. The PI is encouraged to review the eCRFs, working closely with CTMS to ensure prospectively that all required items are appropriately captured in the eCRFs prior to study activation. CTMS will prepare the eCRFs with built-in edit checks to the extent possible to promote data integrity.

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

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

13.3 CTEP Multicenter Guidelines

N/A

13.4 Collaborative Agreements Language

The agent(s) supplied by CTEP, DCTD, NCI used in this protocol is/are provided to the NCI under a Collaborative Agreement (CRADA, CTA, CSA) between the Pharmaceutical Company(ies) (hereinafter referred to as "Collaborator(s)") and the NCI Division of Cancer Treatment and Diagnosis. Therefore, the following obligations/guidelines, in addition to the provisions in the "Intellectual Property Option to Collaborator" (http://ctep.cancer.gov/industryCollaborations2/intellectual_property.htm) contained within the terms of award, apply to the use of the Agent(s) in this study:

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

2. For a clinical protocol where there is an investigational Agent used in combination with (an)other Agent(s), each the subject of different Collaborative Agreements, the access to and

use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-Party Data"):

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

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

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

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

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

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

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

Email: ncicteppubs@mail.nih.gov

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

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APPENDIX A PERFORMANCE STATUS CRITERIA

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

APPENDIX B PATIENT DRUG INFORMATION HANDOUT AND WALLET CARD

Information for Patients, Their Caregivers, and Non-Study Healthcare Team on Possible Interactions with Other Drugs and Herbal Supplements

The patient _____ is enrolled on a clinical trial using the experimental study drug, olaparib in combination with an oral chemotherapy drug, 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.

These are the things that you as a healthcare provider need to know:

Olaparib interacts with certain specific enzymes in the liver and can affect the ability to process other medications, and other medications can affect the ability of your body to process olaparib.

Olaparib interacts with a certain specific enzyme in your liver called CYP 3A4 isoenzymes. The drug itself induces the CYP 3A4 isoenzymes, and olaparib is broken down by these enzymes and will be affected by other drugs that inhibit or induce this enzyme.

To the patient: Take this paper with you to your medical appointments and keep the attached information card in your wallet.

Olaparib may interact with other drugs which can cause side effects. For this reason, it is very important to tell your study doctors of any medicines you are taking before you enroll onto this clinical trial. It is also very important to tell your doctors if you stop taking any regular medicines, or if you start taking a new medicine while you take part in this study. When you talk about your current medications with your doctors, include medicine you buy without a prescription (over-the-counter remedy), or any herbal supplements such as St. John's Wort. **Grapefruit and grapefruit juice are prohibited** while taking olaparib; Seville oranges and Seville orange juice should be avoided. It is helpful to bring your medication bottles or an updated medication list with you.

Many health care providers can write prescriptions. You must tell all of your health care providers (doctors, physician assistants, nurse practitioners, pharmacists) you are taking part in a clinical trial.

These are the things that you and they need to know:

- Olaparib must be used very carefully with other medicines that use liver enzymes. Before you enroll onto the clinical trial, your study doctor will work with your regular health care providers to review any medicines and herbal supplements that are considered strong inducers/inhibitors or substrates of CYP 3A4 isoenzyme.
- Your regular health care provider should check a frequently updated medical reference or call your study doctor before prescribing any new medicine or discontinuing any medicine. Your study doctor's name is

_____

and he or she can be contacted at

_____.

STUDY DRUG INFORMATION WALLET CARD

You are enrolled on a clinical trial using the experimental study drug AZD2281 (Olaparib)**.** This clinical trial is sponsored by the NCI. AZD2281 (Olaparib) may interact with drugs that are processed by your liver, or use certain transport proteins in your body or affects the electrical activity of your heart. Because of this, it is very important to:

- Tell your doctors if you stop taking any medicines or if you start taking any new medicines.
- Tell all of your health care providers (doctors, physician assistants, nurse practitioners, or pharmacists) that you are taking part in a clinical trial.
- Check with your doctor or pharmacist whenever you need to use an over-the-counter medicine or herbal supplement.
- AZD2281 (Olaparib) interacts with a specific liver enzyme called CYP3A4, and must be used very carefully with other medicines that interact with this enzyme. Avoid grapefruit/grapefruit juice.
- Before you enroll onto the clinical trial, your study doctor will work with your regular health care providers to review any medicines and herbal supplements that are considered strong inducers/inhibitors or substrates of CYP3A4.
- Before prescribing new medicines, your regular health care providers should go to a frequently-updated medical reference for a list of drugs to avoid, or contact your study doctor.
- Your study doctor's name is _____

  and can be contacted at _____.

APPENDIX C PATIENT INFORMATION SHEET ON DIARRHEA MANAGEMENT

Diarrhea is a common problem experienced by many patients and is a risk with olaparib. If it is not controlled quickly, it can lead to dehydration.

WHEN TO CALL YOUR DOCTOR TO REPORT DIARRHEA

- Fever 100.5° or higher with diarrhea.
- If you are experiencing diarrhea for the first time after starting therapy. Based on questions answered during that phone call, we will advise starting with 2 milligrams (mg) of loperamide (Imodium) if it seems the symptoms are related to the treatment.
- If you still have diarrhea 24 hours or more than 6 loose bowel movements after starting loperamide (Imodium) (your doctor may advise additional medications or want to evaluate you in person if there is a concern that you are becoming dehydrated).

OVER THE COUNTER MEDICATION MANAGEMENT OF DIARRHEA

- For diarrhea that occurs more than 2 episodes a day, use loperamide (**Imodium**). We recommend that you have loperamide (Imodium) on hand at home before starting therapy.
    - 1st episode of diarrhea: Take 2 caplets (4 mg).
    - During the day: Take 1 caplet (2 mg) after each episode of diarrhea.
    - During the night: Take 2 caplets (4 mg) at bedtime if you are still having diarrhea.
    - Do not take more than 8 tablets (16 mg) of loperamide in 24 hours.

DRINK PLENTY OF FLUIDS

- Drink 8 to 10 large glasses of liquids a day to replace those lost by diarrhea. Drink small quantities at a time slowly.
- **AVOID** caffeinated, very hot, or very cold fluids.
  Examples of acceptable fluids:
    - **Water** (should only be part of the 8 to 10 glasses a day)
    - **Jello/gelatin**
    - **Gatorade**
    - **Clear soup** or **broth**
    - Other non-caffeinated fluids

EAT SMALL MEALS OFTEN

- A good choice of foods for diarrhea is the BRAT diet:
    - **B** - bananas
    - **R** - rice
    - **A** - applesauce
    - **T** - toast
- When these foods are being well tolerated, then you can add other bland low fiber foods such as:
    - Chicken (white meat without the skin), steamed rice, crackers, white bread, pasta noodles without sauce, and canned or cooked fruits without skins.
    - Foods high in potassium: bananas, apricots without skin, peach nectar, potatoes

without skin, broccoli, halibut, mushrooms, asparagus, non-fat milk.

- Foods that can make diarrhea and cramping worse:
  - Fatty, fried, greasy, or spicy foods can cause more problems and discomfort.
  - High-fiber foods: Bran, whole grain cereals, dried fruit, fruit skins, popcorn, nuts, and vegetables.
  - Foods that cause gas: Beer, beans, cabbage, carbonated drinks.

APPENDIX D PATIENT MEDICATION DIARY - OLAPARIB

CTEP-assigned Protocol # 10250
Local Protocol # _____

PATIENT'S MEDICATION DIARY

Today's date _____ **Agent** _____**Olaparib**_____

Patient Name_____ (*initials acceptable*) **Patient Study ID**_____

INSTRUCTIONS TO THE PATIENT:

1. Complete one form every three weeks.
2. You will take ___ tablets each day, ___ in the morning and ___ in the evening. You should take the tablets with 8 oz. water. Do not consume grapefruit, grapefruit juice, or Seville oranges while on olaparib therapy. You can take the morning dose of olaparib together with the dose of temozolomide.
3. If vomiting occurs shortly after the olaparib tablets are swallowed, you should only retake the dose if the intact tablets can be seen and counted.
4. If you miss a scheduled dose for whatever reason (*e.g.*, as a result of forgetting to take the tablets or vomiting), you can take the scheduled dose up to a maximum of 2 hours late. If it is over 2 hours after the scheduled time, skip the dose. Do not double the dose at the next scheduled time to make up for the missed dose.
5. Record the date, the number of tablets you took, and when you took them.
6. If you have any comments or notice any side effects, please record them in the Comments column.
7. Please return the forms to your physician when you go for your next appointment.

Day	Date	Time of morning dose	# of tablets taken	Time of evening dose	# of tablets taken	Comments
1						
2						
3						
4						
5						
6						
7						
8	Do not take olaparib days 8-21					
9						
10						
11						
12						
13						
14						
15						
16						
17						
18						
19						
20						
21						

Physician's Office will complete this section:
Date patient started protocol treatment_____
Date patient was removed from study_____
Patient's planned total daily dose_____
Total number of tablets taken this month _____
Physician/Nurse/Data Manager's Signature _____

Patient's Signature_____

APPENDIX E PATIENT MEDICATION DIARY – TEMOZOLOMIDE

CTEP-assigned Protocol # 10250

Local Protocol # _____

PATIENT'S MEDICATION DIARY

Today's date _____ **Agent** _____**Temozolomide**_____

Patient Name_____ (*initials acceptable)* **Patient Study ID** _____

INSTRUCTIONS TO THE PATIENT:

1. Complete one form every three weeks.
2. You will take ___ capsules each day. You should take the capsules with 8 oz. water, without any food.
3. You should take ondansetron 8 mg immediately before temozolomide to help prevent nausea/vomiting, unless told not to.
4. If vomiting occurs shortly after the temozolomide capsules are swallowed, you should not retake the dose.
5. If you miss a scheduled dose for whatever reason (*e.g.*, as a result of forgetting to take the capsules), you can take the scheduled dose up to a maximum of 12 hours late. If it is over 12 hours after the scheduled time, skip the dose. Do not double the dose at the next scheduled time to make up for the missed dose.
6. Record the date, the number of capsules you took, and when you took them.
7. If you have any comments or notice any side effects, please record them in the Comments column.
8. Please return the forms to your physician when you go for your next appointment.

Day	Date	Did you take ondansetron 8 mg PO prior to your dose?	What time was dose taken?	# of capsules taken	Comments
1					
2					
3					
4					
5					
6					
7					
8	Do not take temozolomide days 8-21.				
9					
10					
11					
12					
13					
14					
15					
16					
17					
18					
19					
20					
21					

Physician's Office will complete this section:

1. Date patient started protocol treatment_____
2. Date patient was removed from study_____

3. Patient's planned total daily dose_____
4. Total number of pills taken this month (each size)_____
5. Physician/Nurse/Data Manager's Signature _____

Patient's Signature:_____

APPENDIX F ACTIONS REQUIRED IN CASES OF COMBINED INCREASE OF AMINOTRANSFERASE AND TOTAL BILIRUBIN – HY'S LAW

Briefly, Hy's Law cases have the following three components:

The drug causes hepatocellular injury, generally shown by a higher incidence of 3-fold or greater elevations above the ULN of ALT or AST than the (non-hepatotoxic) control drug or placebo

Among trial subjects showing such AT elevations, often with ATs much greater than 3xULN, one or more also show elevation of serum TBL to >2xULN, without initial findings of cholestasis (elevated serum ALP)

No other reason can be found to explain the combination of increased AT and TBL, such as viral hepatitis A, B, or C; preexisting or acute liver disease; or another drug capable of causing the observed injury

Finding one Hy's Law case in the clinical trial database is worrisome; finding two is considered highly predictive that the drug has the potential to cause severe drug induced liver injury (DILI) when given to a larger population.

The following actions are required in cases of combined increase of aminotransferase and total bilirubin:

1. Confirmation

In general, an increase of serum AST/A:T to >3xULN should be followed by repeat testing within 48 to 72 hours of all four of the usual serum measures (ALT, AST, ALP, and TBL) to confirm the abnormalities and to determine if they are increasing or decreasing. There also should be inquiry made about symptoms. Serum AT may rise and fall quite rapidly, and waiting a week or two before obtaining confirmation of elevations may lead to a false conclusion that the initially observed abnormality was spurious. Of greater concern, delay in retesting may allow progression to severe worsening if the initial abnormality was the herald of a severe reaction to follow. The need for prompt repeat testing is especially great if AST/ALT is much greater than 3xULN and/or TBL is greater than 2xULN. For outpatient trials, or trials in which subjects are far away from the trial site, it may be difficult for the subjects to return to the trial site promptly. In this case, the subjects should be retested locally, but normal laboratory ranges should be recorded, results should be made available to trial investigators immediately, and the data should be included in the case reports. If symptoms persist or repeat testing shows AST/ALT >3xULN for subjects with normal baseline measures or 2-fold increases above baseline values for subjects with elevated values before drug exposure, it is appropriate to initiate close observation to determine whether the abnormalities are improving or worsening. If close monitoring is not possible, the drug should be discontinued.

2. Close Observation

It is critical to initiate close observation immediately upon detection and confirmation of early signals of possible DILI, and not to wait until the next scheduled visit or monitoring interval. A threshold of aminotransferase levels greater than 3xULN seems reasonable, as lesser elevations are common and nonspecific. If additional testing, beyond that specified in the trial protocol, is carried out, it is important that the subject's information be added to the case report forms and database.
Close observation includes:

Repeating liver enzyme and serum bilirubin tests two or three times weekly. Frequency of retesting can decrease to once a week or less if abnormalities stabilize or the trial drug has been discontinued and the subject is asymptomatic.
Obtaining a more detailed history of symptoms and prior or concurrent diseases.
Obtaining a history of concomitant drug use (including nonprescription medications and herbal and dietary supplement preparations), alcohol use, recreational drug use, and special diets.
Ruling out acute viral hepatitis types A, B, C, D, and E; autoimmune or alcoholic hepatitis; NASH; hypoxic/ischemic hepatopathy; and biliary tract disease.
Obtaining a history of exposure to environmental chemical agents.
Obtaining additional tests to evaluate liver function, as appropriate (e.g., INR, direct bilirubin).
Considering gastroenterology or hepatology consultations.

3. Decision to Stop Drug Administration

It has been observed that de-challenge (stopping drug administration) does not always result in immediate improvement in abnormal lab values. Abnormal test values and symptoms may progress for several days or even weeks after discontinuation of the drug that caused the abnormality. For example, rising TBL usually follows serum AT increases by a few days to weeks. The primary goal of close observation is to determine as quickly as possible whether observed abnormal findings are transient and will resolve spontaneously or will progress. For most DILI, no specific antidotes are available (except N-acetylcysteine for acute acetaminophen overdose if given promptly, and, possibly, intravenous carnitine for valproic acid hepatotoxicity).

Promptly stopping the offending drug usually is the only potentially effective therapy.

Because transient fluctuations of ALT or AST are common, and progression to severe DILI or acute liver failure is uncommon, automatic discontinuation of trial drug upon finding a greater than 3xULN elevation of ALT or AST may be unnecessary. For most people, the liver appears capable of adapting to injury by foreign chemical substances, which may render a person tolerant to the drug despite continued exposure. Stopping a drug at the first hint of mild injury does not permit learning whether adaptation will occur, as it does for drugs such as tacrine, which cause liver injury but do not cause severe DILI. On the other hand, continuing drug appears unacceptably dangerous if there is marked serum aminotransferase elevation or evidence of functional impairment, as indicated by rising bilirubin or INR, which represent substantial liver injury. Although there is no published consensus on exactly when to stop a drug in the face of laboratory abnormalities and the decision will be affected by information on related drugs, the

accumulating clinical experience, the clinical status of the patient, and many other factors, the following can be considered a basic guide. Discontinuation of treatment should be considered if:

ALT or AST >8xULN
ALT or AST >5xULN for more than 2 weeks
ALT or AST >3xULN and (TBL >2xULN or INR >1.5)
ALT or AST >3xULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%)

It should be noted that although these guidelines have not been evaluated systematically in a prospective fashion, they represent an approach that is similar to current practice.

4. Evaluating Data for Alternative Causes

An important purpose of close observation is to gather additional clinical information to seek other possible causes of the observed liver test abnormalities, such as one of the following common causes:

Acute viral hepatitis. The usual onset of hepatocellular DILI is indistinguishable from acute viral hepatitis A or B. Hepatitis C is much less often acute in its onset and tends to be insidious, but it sometimes can resemble acute DILI. The presence of acute viral hepatitis A, B, and C should be evaluated by serological markers. Viral hepatitis D (requires concomitant hepatitis B infection) and E are relatively rare in the United States. Hepatitis E is more common in developing countries, including Southeast Asia, and should be considered in recent travelers to those countries and in patients in trials conducted in those countries. Also rare are hepatocellular liver injuries caused by Epstein-Barr virus, cytomegalovirus, herpes simplex virus, toxoplasmosis, varicella, and parvovirus, although these infections are seen more typically in immuno-suppressed individuals. Adolescent and young adult patients with possible DILI should be tested for Epstein-Barr virus. Hepatitis is common among transplant patients with cytomegalovirus disease.

Alcoholic and autoimmune hepatitis. Acute alcoholic hepatitis usually is recurrent, with a history of binging exposure to alcohol preceding episodes, and it has some characteristic features, such as associated fever, leukocytosis, right upper quadrant pain and tenderness, hepatomegaly, and AST >ALT, that may help distinguish it from other causes of liver injury. Other features of the physical examination may include the presence of stigmata of cirrhosis, such as spider nevi, palmar erythema, estrogenic changes in males, and Dupuytren's contractures. Alcoholic and autoimmune hepatitis should be assessed by history, physical examination, and laboratory testing, including serologic testing (e.g., antinuclear or other antibodies).

Hepatobiliary disorders. Biliary tract disease, such as migration of gallstones or intrahepatic lesions, more often causes cholestatic injury initially and should be investigated with gall bladder and ductal imaging studies, especially if ALP is increased. Malignant interruption of the biliary tract also should be considered.

NASH. NASH may be seen in obese, hyperlipoproteinemic, and/or diabetic patients and may be associated with fluctuating aminotransferase levels, and hepatic and sometimes splenic enlargement. It is sometimes associated with cirrhosis and portal hypertension.

Cardiovascular causes. Cardiovascular disease, especially right heart failure and hypotension or any cause of impaired oxygenation of the liver, may cause acute centrilobular hypoxic cell necrosis (ischemic hepatitis) with rapid and sometimes spectacular increases of serum AT (e.g., AT >10,000 U/L). Cardiovascular dysfunction or impaired liver oxygenation, including hypotension or right heart failure, should be assessed by physical examination and history.

Concomitant treatments. It is critical to discover concomitant treatments, including exposure to nonprescription and dietary supplement products that might be responsible for injury. Many people take multiple drugs, perhaps less often in controlled clinical trials because of exclusion criteria, but subjects may not report taking disallowed drugs or other agents. The possible exposure to potentially toxic herbal or dietary supplement mixtures (sometimes of unknown composition), nonprescription medications such as acetaminophen, or to occupational chemical agents may not be volunteered unless subjects are specifically questioned.

5. Follow-Up to Resolution

All trial subjects showing possible DILI should be followed until all abnormalities return to normal or to the baseline state. DILI may develop or progress even after the causative drug has been stopped. Results should be recorded on the case report form and in the database. Note that longer follow-up can sometimes reveal an off-drug repetition of what had appeared to be DILI, indicating that liver injury was related to underlying liver disease.

6. Re-challenge

Whether or not to re-challenge a subject who showed mild DILI is a difficult decision. Re-exposure may initiate a sometimes explosive and more severe reaction, as was observed with halothane several decades ago. Some cases of DILI show indicators of immunological reaction such as eosinophilia, rash, fever, or other symptoms or findings, and it is possible that such cases are more prone to recur with re-exposure. Re-challenge may not be considered negative unless the subject is exposed to and tolerates the same dose and treatment duration that preceded the original reaction. A negative re-challenge does not necessarily allow a conclusion that the drug did not cause the injury. Most people can adapt to xenobiotic substances, including new drugs, and develop tolerance for them. This has been observed even for drugs that can cause severe injury, such as isoniazid. The large majority of people showing hepatocellular injury while taking isoniazid recover fully or recover while continuing to take the drug, and some, but not all, can resume or continue taking the drug without further adverse consequence. If such tolerance has developed, the use of re-challenge to verify drug causation would give a false negative result.

Generally, re-challenge of subjects with significant AT elevations (>5xULN) should not be attempted. If such subjects are re-challenged, they should be followed closely. Re-challenge can be considered if the subject has shown important benefit from the drug and other options are not available or if substantial accumulated data with the test drug do not show a potential for severe

injury. The subject should be made aware of the potential risk, and consent to the re-challenge, and the PI consulted.

APPENDIX G ACCEPTABLE BIRTH CONTROL METHODS

Women of childbearing potential and their partners, who are sexually active, must agree to the use of two highly effective forms of contraception in combination as listed below. This should be started from the signing of the informed consent and continue throughout the period of taking study treatment and for at least 1 month after last dose of study drugs, or they must totally/truly abstain from any form of sexual intercourse (see below).

Acceptable Non-hormonal birth control methods include:

- Total/True abstinence: When the patient refrains from any form of sexual intercourse and this is in line with their usual and/or preferred lifestyle; this must continue for the total duration of the trial and for at least 1 month after the last dose of study drug. [Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods, or declaration of abstinence solely for the duration of a trial) and withdrawal are not acceptable methods of contraception]
- Vasectomised sexual partner PLUS male condom. With participant assurance that partner received post-vasectomy confirmation of azoospermia.
- Tubal occlusion PLUS male condom
- IUD PLUS male condom. Provided coils are copper-banded

Acceptable hormonal methods:

- Normal and low dose combined oral pills PLUS male condom
- Cerazette (desogestrel) PLUS male condom. Cerazette is currently the only highly efficacious progesterone based pill.
- Hormonal shot or injection (eg., Depo-Provera) PLUS male condom
- Etonogestrel implants (e.g., Implanon, Norplant) PLUS male condom
- Norelgestromin / EE transdermal system PLUS male condom
- Intrauterine system [IUS] device (*eg*., levonorgestrel releasing IUS -Mirena®) PLUS male condom
- Intravaginal device (e.g., EE and etonogestrel) PLUS male condom

APPENDIX H SCHEMA OF BIOMARKER SAMPLE COLLECTION AND PROCESSSING