NCI Protocol #: 10389

Local Protocol #: *TBD*

ClinicalTrials.gov Identifier: *NCT04460937*

TITLE: A Phase 1 Trial Combining WEE1 Inhibitor Adavosertib (AZD1775) with Radiation Therapy for Metastatic or Inoperable and Ineligible for Definitive Chemoradiation Esophageal and Gastroesophageal Junction Cancer

Corresponding Organization: **LAO-OH007** / Ohio State University Comprehensive Cancer Center LAO

Principal Investigator:
Eric D. Miller, M.D., Ph.D.
Ohio State University Comprehensive Cancer Center
460 W 10th Ave
Room A209
Columbus, OH 43210
614-685-4922
614-293-4044
Eric.Miller@osumc.edu

Translational PI:
Terence M. Williams, M.D., Ph.D.
City of Hope Comprehensive Cancer Center
1500 E. Duarte Rd
Radiation Oncology
Duarte, CA 91010
646-456-6282
626-218-5334
terwilliams@coh.org

Participating Organizations

LAO-11030 / University Health Network Princess Margaret Cancer Center LAO
LAO-CA043 / City of Hope Comprehensive Cancer Center LAO
LAO-CT018 / Yale University Cancer Center LAO
LAO-MA036 / Dana-Farber - Harvard Cancer Center LAO
LAO-MD017 / JHU Sidney Kimmel Comprehensive Cancer Center LAO
LAO-OH007 / Ohio State University Comprehensive Cancer Center LAO
LAO-PA015 / University of Pittsburgh Cancer Institute LAO
LAO-TX035 / University of Texas MD Anderson Cancer Center LAO
LAO-NCI / National Cancer Institute LAO

Gastroenterologist:
Sajid Jalil MBBS, MS,
OSU Comprehensive Cancer Center
410 W 10th Ave
Columbus, OH 43210
614-293-6255
614-293-8518
sajid.jalil@osumc.edu

Statistician:
Lai Wei, Ph.D., M.S.
1800 Cannon Drive, 320H Lincoln Tower
Columbus, OH 43210
614-293-6449
614-293-6902
Lai.Wei@osumc.edu

Study Coordinator:
OSU Multi-Center Trial Program
OSU Comprehensive Cancer Center
Fax: 614-366-6652
OSUCCC-CTO-MCTP@osumc.edu

Responsible Research Nurse:
OSU Multi-Center Trial Program
OSU Comprehensive Cancer Center
Fax: 614-366-6652
OSUCCC-CTO-MCTP@osumc.edu

Responsible Data Manager:
OSU Multi-Center Trial Program
Clinical Trials Office
OSU Comprehensive Cancer CenterFax: 614-366-6652
OSUCCC-CTO-MCTP@osumc.edu

NCI-Supplied Agent: AZD1775 (adavosertib, NSC 751084)

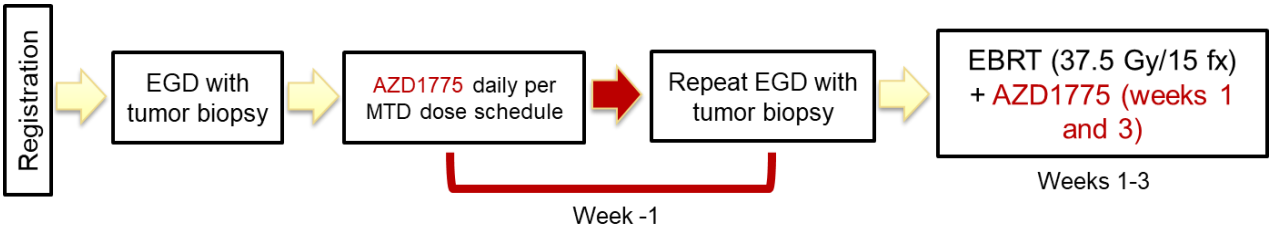
IND #: 122697
IND Sponsor: DCTD, NCI

Protocol Type / Version # / Version Date:
Original / March 23, 2020
Revision 01 / May 7, 2020
Revision 02 / June 8, 2020
Revision 03a / August 3, 2020
Revision 4 / August 26, 2020
Revision 5 / November 18, 2020
Amendment 1 / August 30, 2021
Amendment 2 / December 23, 2021
Amendment 3 / January 13, 2022

SCHEMA

Dose Escalation Schedule		
Dose Level	**Dose**	
	AZD1775 (mg)	**Radiation Therapy (Gy)**
Level -1	150 mg QD on Days 1 and 5 of weeks -1, 1, and 3	37.5 Gy/15 fractions
Level 1*	150 mg QD on Days 1, 3, and 5 of weeks -1, 1, and 3	37.5 Gy/15 fractions
Level 2	200 mg QD on Days 1, 3, and 5 of weeks -1, 1, and 3	37.5 Gy/15 fractions
Level 3	200 mg QD on Days 1-5 of weeks -1, 1, and 3	37.5 Gy/15 fractions
* *Starting dose*		

Expansion Cohort



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1. OBJECTIVES

1.1 Primary Objectives

1.1.1 To identify the maximally tolerated dose of AZD1775 to be used in combination with radiation therapy for patients with esophageal/GEJ cancer that is metastatic or inoperable and not eligible for definitive chemoradiation.

1.2 Secondary Objectives

1.2.1 To observe and record anti-tumor activity. Although the clinical benefit of this drug has not yet been established, the intent of offering this treatment is to provide a possible therapeutic benefit, and thus the patient will be carefully monitored for tumor response and symptom relief in addition to safety and tolerability.

1.2.2 To evaluate the efficacy of AZD1775 when administered in combination with radiation therapy by assessing changes in Ogilvie dysphagia score following treatment, time to second intervention for dysphagia, and overall survival.

1.2.3 To identify biomarkers that are predictive for response to experimental therapy

2. BACKGROUND

2.1 Esophageal Cancer

Esophageal cancer, including gastroesophageal junction (GEJ) cancer, is the 8th most common cancer worldwide and the 6th leading cause of cancer-related deaths (Ferlay *et al*., 2015). Globally, while esophageal squamous cell carcinoma remains the predominant histology in Asia, Africa, and South America, the incidence of esophageal adenocarcinoma has surpassed that of squamous cell carcinoma in North America, Australia, and Europe with predictions that by 2030 up to 1 in 100 men will be diagnosed with esophageal adenocarcinoma during their lifetime (Arnold *et al*., 2017). In the U.S., the incidence of cancers of the esophagus and gastroesophageal junction has increased dramatically in recent decades, largely driven by the rising incidence of adenocarcinoma (Thrift *et al*., 2016). An estimated 17,000 new cases were diagnosed in 2018 with nearly 16,000 deaths (Siegel *et al*., 2018). Nearly 50-60% of patients present with inoperable disease due to tumor invasion of adjacent structures, evidence of distant metastasis, or because the patient is medically unfit for surgery (van Rossum *et al*., 2018).

For patients with metastatic esophageal cancer or who are inoperable and not eligible for definitive chemoradiation, palliative radiation therapy is an established treatment for relieving dysphagia and improving quality of life. However, for patients with localized disease who are not eligible for systemic therapy, external beam radiation therapy alone is unlikely to provide sustained locoregional control (Zhu *et al*., 2015). Local recurrence or progression may result in dysphagia, gastrointestinal bleeding, and esophago-respiratory fistula, resulting in substantial morbidity with subsequent death. For patients with metastatic disease, the development of more effective systemic therapies, including targeted agents and immunotherapy, has made durable control of the

primary tumor a higher priority (Bang *et al*., 2010; Shah *et al*., 2018). Aggressive control of the primary tumor in patients with metastatic esophageal cancer has been associated with improved survival (Guttmann *et al*., 2017). Strategies aimed at improving local control of the primary tumor in patients with metastatic disease or those who are inoperable and not eligible for definitive chemoradiation will certainly have an impact on quality of life and may even impact survival.

2.2 CTEP IND Agent

2.2.1 AZD1775

2.2.1.1 Mechanism of Action

Adavosertib, also known as AZD1775 (previously known as MK-1775), is an inhibitor of WEE1, a protein tyrosine kinase. WEE1 phosphorylates and inhibits cyclin-dependent kinases 1 (CDK1) and 2 (CDK2), and is involved in regulation of the intra-S and G2 cell cycle checkpoints. Proper functioning of these checkpoints is essential for deoxyribonucleic acid (DNA) metabolism and the DNA damage response (Coleman and Dunphy 1994, Parker and Piwnica-Worms 1992). WEE1 overexpression has been demonstrated in hepatocellular carcinoma (Masaki *et al*., 2003), luminal and HER-2 positive breast cancers (Iorns *et al*., 2009), colon, lung carcinoma, and seminoma tumor samples (Mir *et al*., 2010).

CDK1 (also called cell division cycle 2, or CDC2) activity drives a cell from the G2 phase of the cell cycle into mitosis (Investigator's Brochure 2020). In response to DNA damage, WEE1 inhibits CDK1 to prevent the cell from dividing until the damaged DNA is repaired (G2 checkpoint arrest). Inhibition of WEE1 is expected to release a tumor cell from DNA damage-induced arrest at the G2/M boundary, so that un-repaired DNA damage may be taken into mitosis (M-phase). Since cancer cells exhibit higher levels of endogenous damage than normal cells, as well as exhibiting loss of one or more DNA damage response (DDR) capabilities, this is expected to preferentially enhance cancer cell death through mitotic catastrophe compared to normal cells. *In vitro* experiments demonstrate that AZD1775 has synergistic cytotoxic effects when administered in combination with various DNA damaging agents that have divergent mechanisms of action. Therefore, the clinical development of AZD1775 includes its use as a chemosensitizing drug in combination with a cytotoxic agent (or combination of agents) for treatment of advanced solid tumors.

CDK2 activity drives a cell into, and through the DNA synthesis (S-phase) of the cell cycle where the genome is duplicated in preparation for cell division (Investigator's Brochure 2020). An important aspect of CDK2 regulation of replication is the control of replication origin firing. WEE1, under normal circumstances, regulates CDK2 to provide sufficient time to generate the optimum environment for DNA synthesis, such as the generation of a sufficient pool of deoxynucleotide triphosphates (dNTPs), the building blocks of DNA. Inhibition of WEE1 is therefore expected to cause aberrantly high CDK2 activity in S-phase cells that will have multiple consequences for cancer cells that already have G1/S checkpoint aberrations such as p53 mutations or cyclin dependent kinase inhibitor 2A (CDKN2A) deletions (Sherr 1996). These include de-regulated replication origin firing before sufficient dNTPs are available, resulting in a higher degree of replication stress (already greater in cancer cells). The resulting increase in levels of

replication stress will result in replication fork stalling, the generation of unstable DNA replication structures and ultimately replication fork collapse where endonucleases generate DNA damage. Therefore, it is anticipated that AZD1775 will have independent anti-tumor activity in the absence of added chemotherapy, particularly in cancer cells that already have significantly higher levels of replication stress.

Since the majority (if not all) of human cancers harbor abnormalities in their p53 G1/S checkpoint control, they become more dependent on S- and G2- phase checkpoints (Sherr 1996). Thus, S- and G2-checkpoint abrogation caused by AZD1775 may selectively sensitize p53-deficient cells to anti-cancer agents (Wang *et al.,* 2001) while single-agent activity may be seen in cancers with sufficiently high levels of replication stress and endogenous DNA damage.

AZD1775 is a small molecule inhibitor of Wee1 kinase (IC_{50} = 5.18 nM), that directly inhibits phosphorylation of CDC2 at Tyr15 (Investigator's Brochure, 2020). A comprehensive review of AZD1775 can be found in the AZD1775 Investigator's Brochure (2020). AZD1775 has demonstrated activity in multiple cancers based on *in vitro* activity in NCI 60 cell lines.

2.2.1.2 Preclinical Studies

Early preclinical data demonstrated enhancement of chemotherapeutic effect of AZD1775 in combination with carboplatin, cisplatin, and gemcitabine in p53-deficient colon and lung carcinoma cells (Hirai *et al*., 2009), and with 5-FU in p53-deficient colon and pancreatic cells, but not p53 wild-type colon cancer cells (Hirai *et al*., 2010). The same group also demonstrated objective tumor regression in xenograft models using immunodeficient nude rats subcutaneously bearing p53 mutant colon carcinoma exposed to AZD1775 in combination with gemcitabine, in xenografts bearing breast carcinomas exposed to combination with capecitabine (Hirai *et al*., 2009), and in p53-deficient primary pancreatic cancer xenografts, in combination with gemcitabine (Rajeshkumar *et al*., 2011). Direct target effects were demonstrated in xenografts bearing mutant colon carcinoma where administration of AZD1775 (MK-1775) showed reduced pCDC2 levels in blood and tumor samples (Merck unpublished data).

Single agent activity has been validated both in *in vitro* and *in vivo* studies (Merck unpublished data). Independent evaluation of NCI-60 cell lines and hollow fiber studies support single-agent activity of AZD1775 in several colon cancer, melanoma, ovarian cancer, and lung cancer cell lines. More recent xenograft studies also support single agent activity of AZD1775 in human renal carcinoma cells (Merchant *et. al*, unpublished data), human epidermoid cells (Merck unpublished data), and patient-derived sarcoma samples (Kreahling *et al*., 2012).

2.2.1.3 Non-Clinical Pharmacokinetics

The pharmacokinetics (PK) of AZD1775 were evaluated in male Sprague-Dawley rats and Beagle dogs following intravenous (IV) and oral administration (Investigator's Brochure 2020). AZD1775 exhibited high plasma clearance in rats and dogs (57.3 and 50.8 mL/min/kg, respectively). The terminal half-life ($t_{1/2}$) of the compound was short in both species (1.6 and 1.1 hours in rats and dogs, respectively). The oral bioavailability was 59.7% in rats and 33.6% in

dogs. AZD1775 (1 mcM) was moderately bound to plasma proteins from rat, dog, and human, with the unbound fractions being 23.2, 40.0, and 39.5%, respectively.

2.2.1.4 Metabolism

The major metabolic pathway of AZD1775 in human liver preparations is oxidative metabolism (Investigator's Brochure 2020). Oxidative metabolism of AZD1775 is mediated predominantly by cytochrome P450 (CYP) 3A4 and Flavin Containing Monooxygenase 3 (FMO3). AZD1775 is a weak reversible inhibitor of CYP2C8, CYP2C9, CYP2C19, and CYP3A4. In addition, AZD1775 is a time-dependent inhibitor of CYP3A4.

2.2.1.5 Non-clinical toxicology

The toxicology profile of AZD1775 in rats involved the lymphoid and hematopoietic organs and gastrointestinal tract, and included decreased food consumption, body weight loss, hematologic and serum chemistry changes, decreased urine volume and soft feces (Investigator's brochure, 2020). AZD1775 had no effect on CNS function in mice. The cardiovascular effects of AZD1775 were examined in 5 isoflurane-anesthetized and mechanically vented dogs. At doses of 3 and 10 mg/kg, QTc was marginally prolonged by an average of 5% and 7%, respectively. No QTc changes were observed in the intact dog studies or human studies. Evidence of reversibility of all toxicity changes was generally demonstrated by the end of the 2-week recovery period. In a single dose oral toxicity study, severe irreversible toxicity (mortality) was seen in 1 female out of 10 female rats after a single dose at 300 mg/kg (1800 mg/m^2).

2.2.1.6 Clinical Experience

As of 11 November 2019, a total of 883 patients have been exposed to AZD1775 in AZ-sponsored or Merck-sponsored clinical studies (Investigator's Brochure 2020). In addition, approximately 897 patients have also received AZD1775 as part of externally sponsored scientific research.

These patients include those who have received single doses as high as 1,300 mg of AZD1775 as monotherapy, 325 mg of AZD1775 in a single-dose in combination with chemotherapy, and 325 mg twice a day (BID) in a multiple-dose regimen in combination with chemotherapy.

2.2.1.7 Clinical Efficacy

Of 176 evaluable patients who received AZD1775 (either single or multiple doses) as monotherapy or in combination with gemcitabine, cisplatin, or carboplatin on Study PN001, a partial response (PR) (confirmed and unconfirmed) was observed in 17 (9.7%) patients, and stable disease (SD) was observed in 94 (53.4%) patients (Leijen *et al.*, 2016a). A phase 2 trial of AZD1775 in combination with carboplatin in patients with refractory or resistant TP53-mutated ovarian cancer (Study PN009) has also been performed, yielding a 43% overall response rate (partial response + complete response) (Leijen *et al*., 2016b). Recently, a phase I trial was performed evaluating the addition of AZD1775 to gemcitabine and radiation therapy in patients with locally advanced pancreas cancer. The medial overall survival for all patients was 21.7 months, which is

substantially higher than previous results of gemcitabine-based chemoradiation in this patient group (Cuneo *et al*., 2019).

2.2.1.8 Safety

Based on the preliminary safety data available, the most frequent adverse events (AEs) observed were general disorders and administration site conditions (fatigue and asthenia), musculoskeletal and connective tissue disorders (myalgia), and gastrointestinal disorders (gastrointestinal hemorrhage, lower gastrointestinal hemorrhage, upper gastrointestinal hemorrhage, and stomatitis) (Investigator's Brochure 2020). The single-dose maximum tolerated dose (MTD) for both the gemcitabine and cisplatin combination therapies was 200 mg of AZD1775. Dose-limiting toxicities (DLTs) tended to be hematological in nature in the gemcitabine group and constitutional in the cisplatin group. The single-dose MTD for the combination with carboplatin was 325 mg of AZD1775.

Hematologic DLTs have been observed in the multiple-dose AZD1775 combination treatment groups with gemcitabine and cisplatin (Investigator's Brochure 2020). An MTD of AZD1775 in combination with gemcitabine has been established with an interim dose of 50 mg BID on Day 1, 25 mg BID on Day 2, and 25 mg on Day 3. An attenuated once a day (QD) for 2 days schedule in combination with gemcitabine was evaluated. The most frequent AEs included hematologic toxicity, nausea and vomiting, fatigue, and anorexia (Leijen *et al*., 2016a).

The MTD for combination with cisplatin has been exceeded at the 250 mg dose level, and tolerability of the MTD at 200 mg BID has been confirmed (Investigator's Brochure 2020). DLTs observed in the multiple-dose carboplatin combination have been both hematological and constitutional in nature. The multiple-dose MTD in combination with carboplatin is 225 mg BID. No MTD has been reached for AZD1775 in combination with 5-fluorouracil (5-FU). DLTs of encephalopathy and hyponatremia were observed in the AZD1775 20 mg BID in combination with 1000 mg/m^2 5-FU treatment group. For the combination of AZD1775 with topotecan plus cisplatin in study PN008, toxicities were generally hematological and gastrointestinal in nature.

In study PN009 (Leijen *et al*., 2016b) and the pharmacokinetic sub-study to PN001 (Leijen *et al*., 2016a) which used the pre-market formulation, (both combining 2.5-day BID dosing of AZD1775 with carboplatin) toxicities were not qualitatively different from the ones observed in the carboplatin arm of PN001. However, increased hematological toxicity was observed, which was attributed to a drug-drug interaction with aprepitant (which was administered as anti-nausea medication in these studies). Preliminary PK analyses revealed that co-administration of AZD1775 and aprepitant resulted in a ~40% increase of AZD1775 exposure.

2.2.1.9 Clinical PKs

Analyses of preliminary PK data from study PN001 show that the area under the plasma concentration-time curve from time 0 to infinity (AUC0-∞) in a single-dose regimen, the AUC from time 0 to trough (12 hours post dose) at steady-state for a BID dosing regimen, and the maximum plasma drug concentration observed (C_{max}) increase approximately in a dose

proportional manner; the time to maximum plasma drug concentration observed (T_{max}) occurs at 1.0 to 8.0 hours and the $t_{1/2}$ ranged between 7.6 and 12.2 hours (Investigator's Brochure 2020).

2.2.1.10 Potential Drug Interactions

The major metabolic pathway of AZD1775 in human liver preparations is oxidative metabolism. Oxidative metabolism of AZD1775 is mediated predominantly by CYP3A4 and FMO3 (Investigator's Brochure 2020). AZD1775 is also a weak reversible inhibitor of CYP2C8, CYP2C9, CYP2C19 and CYP3A4. In addition, AZD1775 is a time-dependent inhibitor of CYP3A4 in *in vitro* human liver microsomes. A formal clinical drug interaction study (D6014C00006) evaluating the effect of AZD1775 on PK of substrates for CYP3A, CYP2C19, and CYP1A2 has been conducted in advanced solid tumor patients. The results of this study suggest that AZD1775 is a weak inhibitor of these enzymes and is unlikely to result in clinically significant drug interaction.

2.3 Rationale

Radiation therapy induces DNA damage, resulting in activation of apoptotic pathways or inducing post-mitotic death due to unrepaired damage. Following DNA damage, cells rely on cell cycle checkpoints to provide sufficient time for DNA repair prior to cell division. Esophageal cancer cells often lack a functional G1 checkpoint due to a high frequency of *TP53* mutations. Based on TCGA data, up to 91% of squamous cell and 71% of adenocarcinoma esophageal cancers possess a *TP53* mutation (The Cancer Genome Atlas Research Network, 2017), making them heavily dependent on the G2 checkpoint for DNA damage repair. WEE1, a tyrosine kinase, is phosphorylated following DNA damage and serves as a regulator of the G2 checkpoint and, thus, a regulator of genomic stability by inhibitory phosphorylation of CDK1 resulting in cell cycle arrest and permitting DNA repair prior to proceeding with mitosis (Dominguez-Kelly *et al.*, 2011; Watanabe *et al.*, 1995). Inhibiting WEE1 results in replication stress, loss of genomic integrity, nucleotide shortage, and subsequent double-strand DNA breaks (Beck *et al*., 2012). AZD1775 is a potent and selective small molecule inhibitor of WEE1 kinase and has been shown to sensitize tumor cell lines to both chemotherapy and radiation (Bridges *et al.*, 2011; Hirai *et al.*, 2009; Kausar *et al.*, 2015). *Due to the high incidence of TP53 mutations and the dependence on the WEE1/Chk1 mediated G2-M checkpoint to repair DNA, esophageal/GEJ cancer is a logical site to consider the addition of a WEE1 kinase inhibitor to radiation therapy.*

There are several phase 1 and 2 studies which have been performed using AZD1775 both as monotherapy and in combination with additional chemotherapy agents (Do *et al*., 2015; Leijen *et al*., 2016a; Leijen *et al.*, 2016b; Mendéz *et al*., 2018). Do *et al.* conducted a phase 1 study of single agent AZD1775 for patients with refractory solid tumors (Do *et al*., 2015). The MTD was 225 mg BID orally over 2.5 days per week for 2 weeks per 21-day cycle. The most common toxicities observed were myelosuppression and diarrhea with DLTs including supraventricular tachyarrhythmia and myelosuppression. Leijen *et al.* conducted a phase 1 study evaluating AZD1775 as monotherapy or in combination with gemcitabine, cisplatin, or carboplatin for refractory solid tumors (Leijen *et al*., 2016a). The most common adverse events in the combination regimens consisted of fatigue, nausea/vomiting, diarrhea, and hematologic toxicity. The rate of grade ≥3 gastrointestinal and hematologic toxicity was 17% and 57%, respectively. The MTD for

the combination regimen with carboplatin was 225 mg twice a day for 2.5 days. Of note, the response rate in *TP53*-mutated patient was 21% *vs.* 12% in *TP53* wild-type patients. A subsequent phase 2 study from the same group in patients with *TP53*-mutated ovarian cancer refractory to first-line therapy was performed combining AZD1775 with carboplatin (Leijen *et al*., 2016b). AZD1775 was administered at a dose of 225 mg orally twice daily over 2.5 days every 21 days with carboplatin AUC 5. The overall response rate was 43% with manageable toxicity with the most frequent grade 3 or 4 events being thrombocytopenia (48%) and neutropenia (37%). Mendéz *et al*. performed a phase 1 study for patients with stage III/IVB head and neck squamous cell carcinoma (Mendéz *et al*., 2018). AZD1775 was given BID over 2.5 days during the first week, then in combination with cisplatin/docetaxel for 3 additional weeks. The MTD was 150 mg orally BID for 2.5 days with the only DLT being grade 3 diarrhea. The most common grade ≥2 toxicities were diarrhea, fatigue, and neutropenia. Eight of 10 evaluable patients had a partial response based on pathology and imaging with PET. The safety of AZD1775 in combination with gemcitabine-based chemoradiation for unresectable pancreas adenocarcinoma has been evaluated (Cuneo *et al*., 2019). In this phase 1 trial, AZD1775 was administered orally on Days 1 and 2, and on Days 8 and 9 of a 3-week cycle, gemcitabine 1,000 mg/m^2 was given on Days 1 and 8 of a 3-week treatment cycle, and radiation therapy was administered to a dose of 52.5 Gy in 25 fractions. An AZD1775 dose of 150 mg was determined to be the optimal dose with 24% of patients experiencing a DLT most commonly anorexia, nausea, or fatigue.

In summary, a number of phase 1 trials have been performed investigating the use of the WEE1 inhibitor, AZD1775, either in monotherapy or in combination with multiple chemotherapy regimens. Thus far, AZD1775 appears to be reasonably tolerated with the main toxicities including gastrointestinal, hematologic, and fatigue. Favorable responses have been observed, in particular, tumors with *TP53* mutations. Combining AZD1775 with systemic therapy and radiation therapy has also been safely performed based on recently published data in pancreas cancer (Cuneo *et al*., 2019). We hypothesize that inhibiting WEE1 kinase activity with AZD1775 in combination with targeted radiation therapy will lead to improved tumor control and response of esophageal/GEJ cancer. Herein, we propose a phase 1 dose escalation study to evaluate the safety of adding AZD1775 to radiation therapy for patients with metastatic or inoperable and ineligible for definitive chemoradiation esophageal/GEJ cancer.

2.4 Correlative Studies Background

2.4.1 Integrated Studies

2.4.1.1 Whole exome sequencing

Based on data from The Cancer Genome Atlas, esophageal cancer has an extremely high frequency of *TP53* mutation, making these cancers highly dependent on the G2 checkpoint for DNA damage repair. WEE1 is a tyrosine kinase which is phosphorylated following DNA damage and serves as a regulator of the G2 checkpoint by inhibitory phosphorylation of CDK1, resulting in cell cycle arrest and permitting DNA repair prior to proceeding with mitosis. However, it is currently unknown if *TP53* is a potential predictive biomarker of response to treatment with AZD1775 combined with radiation therapy. In addition, it is unknown if there are additional predictive biomarkers of response including DNA repair mutations in *ATM*, *ATR*,

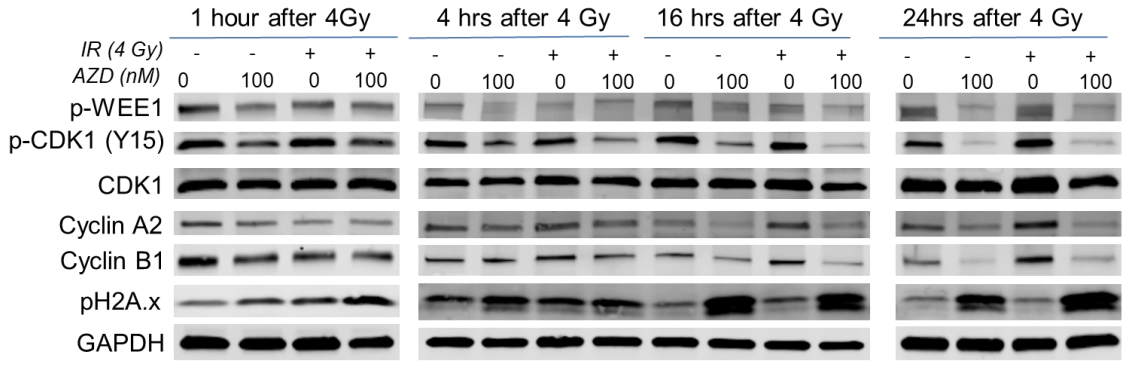
or *ARX*. We hypothesize that mutations in *TP53*, in addition to other DNA repair mutations, will serve as predictive biomarkers of response to treatment with AZD1775 and radiation therapy.

2.4.1.2 ɣH2AX, pNBS1 IFA with βCATN segmentation

Following double-strand DNA breaks, recruitment of MRN complex proteins serves as a sensor of DNA breakage and leads to phosphorylation of NBS1 (pNBS1). pNBS1, in turn, activates protein kinase ATM which subsequently phosphorylates additional substrate proteins, including ɣH2AX which localizes at sites of DNA double-strand breaks. Thus, both pNBS1 and ɣH2AX serve as markers of unrepaired double-strand DNA breaks. We hypothesize that treatment with AZD1775 in the expansion cohort will result in an increase in both pNBS1 and ɣH2AX.

The effects of AZD1775 on esophageal cancer cell cycle markers are demonstrated in **Figure 1** (Yang *et al.*, 2020). FLO-1 esophageal adenocarcinoma cells were treated with 0 nM or 100 nM of AZD1775 alone or in combination with a single exposure of 4 Gy using an RS 2000 biological irradiator with 165 kV X-rays. Cells were pre-treated with the study drug for 2 hours prior to radiation delivery. The cells were then lysed at 1, 4, 16, or 24 hours following radiation exposure and Western blotting was performed to detect alterations in cell cycle markers. As seen in **Figure 1**, AZD1775 effectively blocks WEE1 kinase activity (pWEE1 S642) leading to a decrease in inhibitory p-CDK1 (Y15) levels, suggesting a subsequent increase in CDK1 activity. An increase in pH2A.x was also observed suggesting an increase in unrepaired double-strand DNA breaks.

Figure 1: Effects of AZD1775 on FLO-1 cell cycle markers (Yang *et al.*, 2020).



2.4.2 Exploratory Study

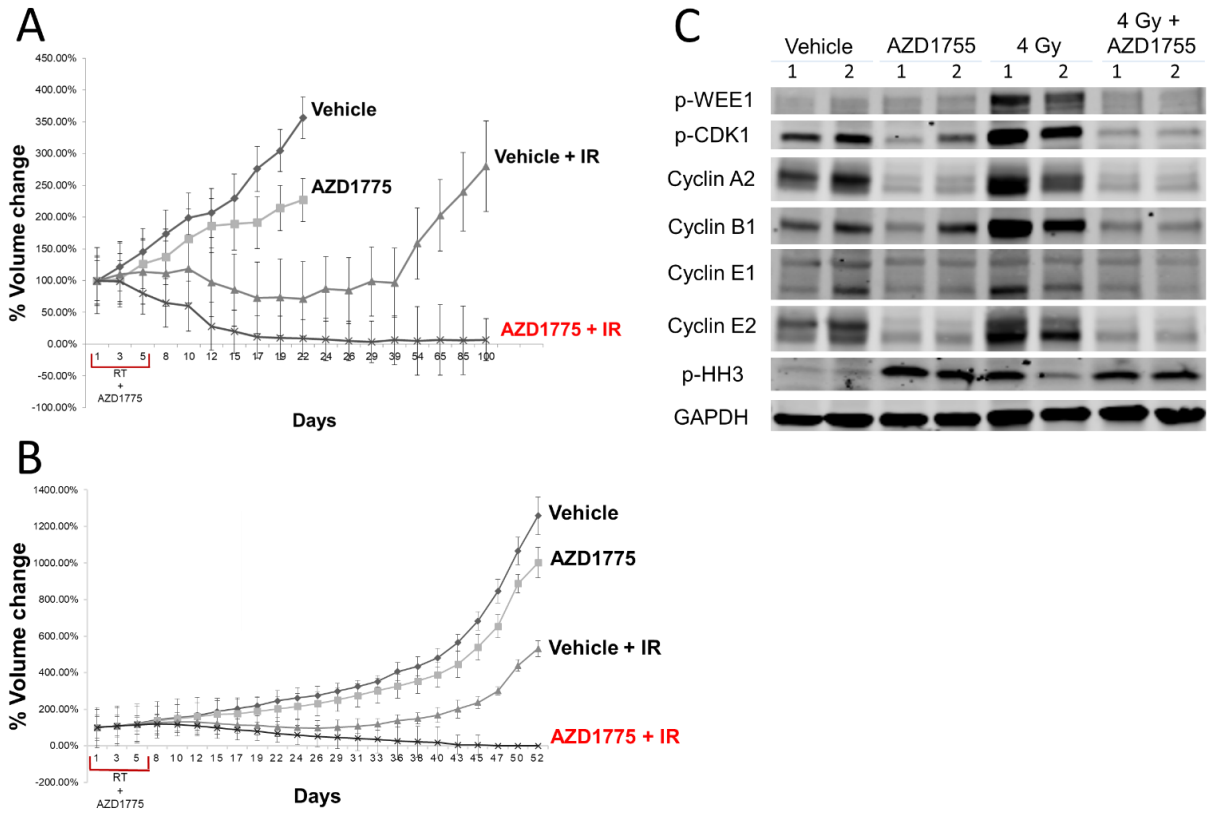
2.4.2.1 p-HH3 (S20), p-cdk1 (Y15), pWEE1(S642), and p-Chk1(S345) IHC

WEE1 mediated phosphorylation and inhibition of CDK1 plays a critical role in G2/M checkpoint during normal cell growth and following DNA damage or replication stress. Following DNA damage or replication stress, activation of CHK1 by ATR phosphorylates and stimulates WEE1 activation to suppress CDK1 activity, which prevents entry into mitosis. Thus, p-cdk1 and pWEE1 serve as markers of WEE1 inhibition, pChk1 serves as a marker of DNA damage response, and

pHH3 serves as a marker for premature mitosis. We hypothesize that treatment with AZD1775 in the expansion cohort will result in a decrease in pWEE1 and p-cdk1 and an increase in pChk1 and p-HH3.

In vivo experiments were performed where 1 to 5 million FLO-1 cells were injected in the left flanks of mice. Once the tumor size reached 100-150 cm^3, the mice were randomized into 4 groups: vehicle, AZD1775 alone, radiation alone, and AZD1775 with radiation with 8-10 mice per group. In the groups that received radiation therapy, 4 Gy per day was delivered for 5 consecutive days. AZD1775 was given 40 mg/kg twice daily for 5 consecutive days via oral gavage (for those mice administered radiation, AZD1775 was given 2 hours before radiation). Tumor size was measured 3 times per week using a caliper and the volume calculated using the formula (L x W x W)/2. A marked enhancement in growth delay in the AZD1775 + radiation group was observed as shown in **Figure 2A**, with no evidence of tumor recurrence in most mice. Similar results were observed in our 2nd *in vivo* model with 15 mice bearing OE33 cell tumors (**Figure 2B**). To study pharmacodynamics, a group of eight mice bearing FLO-1 tumors were also treated with the four combinations for 3 days and the tumors were isolated, lysed, and immunoblotting performed. Western blot results are shown in **Figure 2C** with 2 tumors per group represented (Yang *et al.*, 2020). Radiation (4 Gy) induced pWEE1 and pCDK1, suggestive of induction of the G2 checkpoint response. However, a marked decrease in p-CDK1 (and pWEE1) was seen when comparing the radiation alone to the radiation with AZD1775, as well as reduced expression of cyclins A2, B1, E1, and E2. Lastly, an increase in p-HH3 (which is a marker of premature mitosis) was also observed in the tumors treated with AZD1775 with or without radiation therapy.

Figure 2: Effects of AZD1775 on tumor growth in mice bearing FLO-1 cell tumors (A), OE33 cell tumors (B), and cell cycle markers in excised FLO-1 cell tumors (C) (Yang *et al.*, 2020).



3. PATIENT SELECTION

3.1 Eligibility Criteria

3.1.1 Patients must have histologically confirmed esophageal cancer (either squamous cell or adenocarcinoma), including Siewert gastroesophageal junction adenocarcinomas Types 1 and 2, that is inoperable and not eligible for definitive chemoradiation after multidisciplinary review or have pathologically confirmed or imaging consistent with metastatic disease.

3.1.2 Age ≥18 years. Because no dosing or adverse event data are currently available on the use of AZD1775 in combination with radiation therapy in patients <18 years of age, children are excluded from this study

3.1.3 ECOG performance status 0-1 (Karnofsky ≥70%, see Appendix A).

3.1.4 Patients must have adequate organ and marrow function as defined below:

- leukocytes ≥3,000/mcL
- 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) ≤3 × institutional ULN
- creatinine ≤ 1.5 × institutional ULN
  OR
- glomerular filtration rate (GFR) ≥60 mL /min/1.73 m^2 unless data exists supporting safe use at lower kidney function values, no lower than 30 mL/min/1.73 m^2 (see Appendix B)

3.1.5 For patients with evidence of chronic hepatitis B virus (HBV) infection, the HBV viral load must be undetectable on suppressive therapy, if indicated.

3.1.6 Patients with a history of hepatitis C virus (HCV) infection must have been treated and cured. For patients with HCV infection who are currently on treatment, they are eligible if they have an undetectable HCV viral load.

3.1.7 Patients with **treated brain metastases** are eligible if follow-up brain imaging after central nervous system (CNS)-directed therapy shows no evidence of progression.

3.1.8 Patients with **new or progressive brain metastases** (active brain metastases) or **leptomeningeal disease** are eligible if the treating physician determines that immediate CNS specific treatment is not required and is unlikely to be required during the first cycle of therapy.

3.1.9 Patients with a prior or concurrent malignancy whose natural history or treatment does not have the potential to interfere with the safety or efficacy assessment of the investigational regimen are eligible for this trial.

3.1.10 Patients with known history or current symptoms of cardiac disease, or history of treatment with cardiotoxic agents, should have a clinical risk assessment of cardiac function using the New York Heart Association Functional Classification. To be eligible for this trial, patients should be class 2B or better.

3.1.11 Patients able to swallow whole capsules. Patients with esophageal stents and/or feeding tubes are eligible but must be able to swallow whole capsules. Capsules may not be opened or put down a feeding tube.

3.1.12 Patients with a life expectancy > 3 months.

3.1.13 Patients must have an electrocardiogram (ECG) within 8 weeks prior to treatment assignment and must have no clinically important abnormalities in rhythm, conduction or morphology of resting ECG

3.1.14 a) Resting corrected QTc interval using the Fridericia formula (QTcF) >480 msec (as calculated per institutional standards) obtained from an ECG (Note: if one ECG demonstrates a QTcF >480 msec, then a mean QTcF of ≤480 msec obtained from 3

ECGs 2-5 minutes apart, is required at study entry), or b) patients with congenital long QT syndrome are excluded

3.1.15 The effects of AZD1775 on the developing human fetus are unknown. For this reason and because other therapeutic agents used in this trial are known to be teratogenic, women of child-bearing potential must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) for 2 weeks prior to study drug exposure, the duration of study participation, and for 1 month after completing treatment. Women of child-bearing potential must have a negative serum or urine pregnancy test (minimum sensitivity 25 IU/L or equivalent units of HCG) within 1 week of registration. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately. Men treated or enrolled on this protocol must also agree to use adequate contraception for the duration of study participation and for 3 months after completion of treatment. Male patients should not donate sperm during exposure to study drug and for 3 months after study drug discontinuation.

3.1.16 Ability to understand and the willingness to sign a written informed consent document. Participants with impaired decision-making capacity (IDMC) will not be eligible.

3.2 Exclusion Criteria

3.2.1 Patients who have had chemotherapy or radiotherapy within 3 weeks (6 weeks for nitrosoureas or mitomycin C) prior to starting study therapy.

3.2.2 Patients who have not recovered from adverse events due to prior anti-cancer therapy (*i.e.*, have residual toxicities > Grade 1) with the exception of alopecia.

3.2.3 Patients who are receiving any other investigational agents.

3.2.4 History of allergic reactions attributed to compounds of similar chemical or biologic composition to AZD1775.

3.2.5 Patients receiving any medications or substances that are strong inhibitors or inducers of CYP3A4 are ineligible. 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.

3.2.6 Patients with uncontrolled intercurrent illness.

3.2.7 Patients with psychiatric illness/social situations that would limit compliance with study requirements.

3.2.8 Pregnant women are excluded from this study because AZD1775 is a WEE1 inhibiting agent with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with AZD1775, breastfeeding should be discontinued if the mother is treated with AZD1775. These potential risks may also apply to other agents used in this study.

3.2.9 Prior thoracic or abdominal radiation therapy for cancer that would result in significant overlap of radiation therapy fields at the discretion of the investigator.

3.2.10 Patients with congenital long QT syndrome or with a history of Torsades de pointes unless all risk factors contributed to Torsades have been corrected. AZD1775 has not been studied in patients with ventricular arrhythmias or recent myocardial infarction.

3.2.11 Eligibility of subjects receiving any medications or substances with the potential to affect the activity or pharmacokinetics of AZD1775 will be determined following review by the principal investigator.

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 and National Cancer Institute (NCI) policy require all individuals contributing to NCI-sponsored trials to register and to renew their registration annually. To register, all individuals must obtain a Cancer Therapy Evaluation Program (CTEP) Identity and Access Management (IAM) account at https://ctepcore.nci.nih.gov/iam. In addition, persons with a registration type of Investigator (IVR), Non-Physician Investigator (NPIVR), or Associate Plus (AP) must complete their annual registration using CTEP's web-based Registration and Credential Repository (RCR) at https://ctepcore.nci.nih.gov/rcr.

RCR utilizes five person registration types.

- IVR: MD, DO, or international equivalent,

- NPIVR: advanced practice providers (*e.g.*, NP or PA) or graduate level researchers (*e.g.*, PhD),
- AP: clinical site staff (*e.g.*, RN or CRA) with data entry access to CTSU applications such as the Roster Update Management System (RUMS), OPEN, Rave, acting as a primary site contact, or with consenting privileges,
- Associate (A): other clinical site staff involved in the conduct of NCI-sponsored trials, and
- Associate Basic (AB): individuals (*e.g.*, pharmaceutical company employees) with limited access to NCI-supported systems.

RCR requires the following registration documents:

Documentation Required	IVR	NPIVR	AP	A	AB
FDA Form 1572	✓	✓			
Financial Disclosure Form	✓	✓	✓		
NCI Biosketch (education, training, employment, license, and certification)	✓	✓	✓		
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 Institutional Review Boards (IRBs) covering their practice sites on the FDA Form 1572 in RCR to allow the following:

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

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

Additional information is located 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 Cancer Trials Support Unit (CTSU).

IRB Approval

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

Sites using their local IRB or REB must submit their approval to the CTSU Regulatory Office using the Regulatory Submission Portal located in the Regulatory section of the CTSU website. Acceptable documentation of local IRB/REB approval includes:

- Local IRB documentation,
- IRB-signed CTSU IRB Certification Form, and/or
- Protocol of Human Subjects Assurance Identification/IRB Certification/Declaration of Exemption Form.

In addition, the Site-Protocol PI (*i.e.*, the investigator on the IRB/REB approval) must meet the following five criteria to complete processing of the IRB/REB approval record:

- Holds an active CTEP status,
- Rostered at the site on the IRB/REB approval (*applies to US and Canadian sites only*) and on at least one participating roster,
- If using NCI CIRB, rostered on the NCI CIRB Signatory record,
- Includes the IRB number of the IRB providing approval in the Form FDA 1572 in the RCR profile, and
- Holds the appropriate CTEP registration type for the protocol.

Additional Requirements

Additional requirements to obtain an approved site registration status include:

- An active Federalwide Assurance (FWA) number,
- An active roster affiliation with the Lead Protocol Organization (LPO) or a Participating Organization (PO), and
- Compliance with all protocol-specific requirements (PSRs).

4.2.1 Downloading Regulatory Documents

Download the site registration forms from the protocol-specific page located on the CTSU members' website. Permission to view and download this protocol and its supporting documents

is restricted based on person and site roster assignment. To participate, the institution and its associated investigators and staff must be associated with the LPO or a PO on the protocol. One way to search for a protocol is listed below.

- Log in to the CTSU members' website (https://www.ctsu.org) using your CTEP-IAM username and password,
- Click on *Protocols* in the upper left of the screen
    - Enter the protocol number in the search field at the top of the protocol tree, or
    - Click on the By Lead Organization folder to expand, then select **LAO-OH007**, and protocol number 10389,
- Click on *Documents*, select *Site Registration*, and download and complete the forms provided. (Note: For sites under the CIRB, IRB data will load automatically to the CTSU.)

4.2.2 Protocol Specific Requirements For NCI Protocol #10389 Site Registration

- Specimen Tracking System Training Requirement:
    - 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.
    - Please contact STS Support at Theradex for the training (STS.Support@theradex.com, Theradex phone: 609-799-7580).

4.2.3 Submitting Regulatory Documents

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

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

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

Delegation of Tasks Log (DTL)

Each site must complete a protocol-specific DTL using the DTL application in the Delegation

Log section on the CTSU members' website. The Clinical Investigator (CI) is required to review and electronically sign the DTL prior to the site receiving an approved site registration status and enrolling patients to the study. To maintain an approved site registration status the CI must re-sign the DTL at least annually and when a new version of the DTL is released; and activate new task assignments requiring CI sign-off. Any individual at the enrolling site on a participating roster may initiate the site DTL. Once the DTL is submitted for CI approval, only the designated DTL Administrators or the CI may update the DTL. Instructions on completing the DTL are available in the Help Topics button in the DTL application and include a Master Task List, which describes DTL task assignments, CI signature, and CTEP registration requirements.

4.2.4 Checking Site Registration Status

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

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

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

4.3 Patient Registration

4.3.1 OPEN / IWRS

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

Requirements for OPEN access:

- A valid CTEP-IAM account.
- To perform enrollments or request slot reservations: Must be on an LPO roster, ETCTN corresponding roster, or PO roster with the role of Registrar. Registrars must hold a minimum of an Associate Plus (AP) registration type.
- If a DTL is required for the study, the registrar must hold the OPEN Registrar task on the DTL for the site.
- Have an approved site registration for the protocol prior to patient enrollment.

To assign an Investigator (IVR) or Non-Physician Investigator (NPIVR) as the treating,

crediting, consenting, drug shipment (IVR only), or receiving investigator for a patient transfer in OPEN, the IVR or NPIVR must list the IRB number used on the site's IRB approval on their Form FDA 1572 in RCR. If a DTL is required for the study, the IVR or NPIVR must be assigned the appropriate OPEN-related tasks on the DTL.

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

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

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

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

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

4.3.2 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 Rave user roles: "Rave CRA" and "Rave CRA (Labadmin)" 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 in the Data Management section under the Rave Home tab and then under Rave Resource Materials.
- **Important: Failure to complete required fields in STS may result in a delay in sample processing.** Any case reimbursements associated with sample submissions will not be credited if samples requiring STS submission are not logged into STS.

Detailed instructions on use of the STS can be found in Section 5.4.

4.3.3 OPEN/IWRS Questions?

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

Theradex has developed a Slot Reservations and Cohort Management User Guide, which is available on the Theradex website: http://www.theradex.com/clinicalTechnologies/?National-Cancer-Institute-NCI-11. This link to the Theradex website is also on the CTSU website OPEN tab. For questions about the use of IWRS for slot reservations, contact the Theradex Helpdesk at 609-619-7862 or Theradex main number 609-799-7580; CTMSSupport@theradex.com.

4.4 General Guidelines

Following registration, patients should begin protocol treatment within 28 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:
Archival		
	• Archival formalin-fixed paraffin-embedded (FFPE) tumor tissue block (preferred)[1] (mandatory) If a block is not available, then submit: • 1 H&E stained slide (3-5 µm) • 30-50 unstained, uncharged, air-dried slides (10 µm)	ETCTN Biorepository
Baseline		
	• 10 mL blood in EDTA tube (mandatory)[3] • 2 snap-frozen tumor tissue forceps biopsies (mandatory; expansion cohort only)[2,4] • 2 tumor tissue forceps biopsies in formalin (mandatory where clinically feasible; expansion cohort only)[2]	ETCTN Biorepository
Week -1 Day 4 or 5 (Expansion Cohort Only)		

	• 2 snap-frozen tissue forceps biopsies (mandatory) [2,4] • 2 tumor tissue forceps biopsies in formalin (mandatory where clinically feasible)[2]	ETCTN Biorepository

[1]For archival tissue, a copy of the corresponding anatomic pathology report must be sent with the tissue and uploaded to Rave. If submitting slides, then slides must be processed in order, and numbered sequentially (*e.g.*, H&E stained slide is created first and labeled 1, unstained slides are then created and numbered 2 – 51).
[2]For new biopsies, the Tissue Biopsy Verification Form (Appendix G), a copy of the radiology and/or operative reports from the tissue removal procedure *and* the diagnostic anatomic pathology report must be sent with the tissue to the ETCTN Biorepository.
[3] within 28 days prior to study entry
[4] Refer to Appendix H for detailed SOP on snap frozen tumor tissue

5.2 Summary Tables for Gastroenterologist for Research Biopsies

Biopsy #: 1				
Trial Time Point: Baseline (Expansion cohort only)				
IR Biopsy Definition: Research – No Clinical Impact				
Core Priority	**Use in the Trial**	**Biomarker Name(s)**	**Tumor Content Required**	**Post-Biopsy Processing**
1	Integrated	ɣH2AX, pNBS1 IFA with βCATN segmentation	>50% OR 25-50%	Frozen
2	Exploratory	p-HH3 (S20), p-cdk1 (Y15), pWEE1(S642), andp-Chk1(S345) IHC	Prefer 25-50% or >50%, but 5-25% is acceptable	Formalin

Biopsy #: 2				
Trial Time Point: Week -1 Day 4 or 5 (Expansion cohort only)				
IR Biopsy Definition: Research – No Clinical Impact				
Core Priority	**Use in the Trial**	**Biomarker Name(s)**	**Tumor Content Required**	**Post-Biopsy Processing**
1	Integrated	ɣH2AX, pNBS1 IFA with βCATN segmentation	>50% OR 25-50%	Frozen

2	Exploratory	p-HH3 (S20), p-cdk1 (Y15), pWEE1(S642), andp-Chk1(S345) IHC	Prefer 25-50% or >50%, but 5-25% is acceptable	Formalin

Note: Pre-biopsy assessments will be reported and tracked through a trial-specific Case Report Form (CRF) within the CTEP Medidata Rave system (see Appendix F).

5.3 Specimen Procurement Kits and Scheduling

5.3.1 Specimen Procurement 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 kits per kit type per day (daily max = 6 kits). Kits are shipped ground, so please allow 5-7 days for receipt. A complete list of kit contents for each kit type is located on the Kit Management system website.

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

5.3.2 Scheduling of Specimen Collections

- Tumor tissue specimens collected during biopsy procedures and fixed in formalin must be shipped on the same day of collection.
- Tissue in formalin can be collected Monday through Wednesday and shipped overnight for arrival on Tuesday through Thursday at the ETCTN Biorepository at Nationwide Children's Hospital.
- Specimens submitted as frozen (snap-frozen tissue) can be collected on any day but must be stored frozen and shipped to the ETCTN Biorepository on Monday through Thursday. In the event that frozen specimens cannot be shipped immediately, they must be maintained in a -70°C to -80°C freezer.

- Fresh blood specimens may be collected and shipped Monday through Friday.

5.4 Specimen Tracking System Instructions

5.4.1 Specimen Tracking System Overview and Enrollment Instructions

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

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

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

Immediately following enrollment, the institutional anatomical pathology report for the diagnosis under which the patient is being enrolled must be uploaded into Rave. The report must include the surgical pathology ID (SPID), collection date, block number, and the IWRS-assigned UPID and patient study ID for this trial. For newly acquired biopsies, 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, date of birth, initials, medical record number, and patient contact information from the institutional pathology report prior to submission.**

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

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

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

5.4.2 Specimen Labeling

5.4.2.1 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)

5.4.2.2 Tissue Specimen Labels

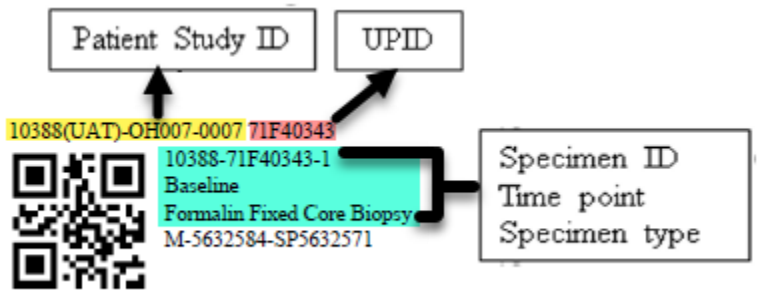
Include the following on all tissue specimens or containers (*e.g.*, formalin jar):

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

5.4.2.3 Example of Specimen Label Generated by STS

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

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



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

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

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

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

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

5.4.3 Overview of Process at Treating Site

5.4.3.1 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.

5.4.3.2 Rave Specimen Tracking Process Steps

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

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

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

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

- Label specimen containers and write collection date on each label. After collection, store

labeled specimens as described in Section 5.4.2.

- 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 Tissue Biopsy 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, date of birth, mailing address, medical record number or social security number (SSN), redacted. Do not redact SPID, block number, diagnosis, or relevant dates (such as collection date), and include the UPID and patient study ID on each document.

Step 3: Complete specimen data entry.

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

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

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

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

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

Step 6: Send email notification.

- For only one of the specimens in the shipment, click "Send Email Alert" checkbox on the **Shipping Status** CRF to email recipient.

Step 7: Ship the specimen(s).

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

5.5 Specimen Collection

5.5.1 Archival or Formalin-Fixed Paraffin-Embedded (FFPE) Tumor Specimen

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

- Tissue must have been collected within 24 months prior to registration

- FFPE tumor tissue block(s) must be submitted. The optimal block is at least 70% tumor. Specimen size requirement is as follows:
    - Surface area: 25 mm^2 is optimal. Minimum is 5 mm^2.
    - Volume: 1 mm^3 optimal. Minimum volume is 0.2 mm^3, however the success of DNA extraction decreases at suboptimal tissue volume.

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

- One (1) H&E slide (3-5 µm)
- Thirty to fifty (30 – 50) 10 µm unstained air-dried uncharged slides

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

See Section 5.4.2 for labeling instructions.

5.5.2 Collection of Snap-Frozen Biopsies

1. Tissue should be frozen as soon as possible. Optimally, freeze within 2 minutes from resection. Refer to Appendix H for detailed SOP.
2. Label cryovial(s) according to instructions in Section 5.4.2.
3. Follow instructions described in Appendix H for snap-freezing tissue, including, placing the tissue in a pre-chilled cryovial and freeze the tube. Keep frozen until shipment to the ETCTN Biorepository. Do not ship to FNLCR.

5.5.3 Formalin-Fixed Tumor Biopsies

1. Label formalin-filled containers according to instructions in Section 5.4.2.
2. Obtain 2-3 tissue forceps biopsy specimens, and place one biopsy 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 package containers into the shipping kit according to instructions in Section 5.6. Keep tissue in formalin jars at room temperature until shipment to the ETCTN Biorepository.

5.5.4 Blood Collection

5.5.4.1 Collection of Blood in EDTA Tubes for Shipping Whole Blood

1. Label EDTA tubes according to the instructions in Section 5.4.2.
2. Collect 10 mL blood in EDTA tube(s) and gently invert tube to mix.
3. Ship on day of collection (whenever possible) according to instructions in Section 5.6.
4. If blood cannot be shipped on the day of collection (e.g., a late scheduled collection), then refrigerate until shipment.

5.6 Shipping Specimens from Clinical Site to the ETCTN Biorepository

5.6.1 General Shipping Information

Core biopsies that are fixed in formalin, snap frozen core biopsies, and fresh blood should be shipped as one shipment in a dual chambered kit, whenever possible. The shipping container 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 ambient side of the shipping container.

For new biopsies, the Tissue Biopsy Verification Form (Appendix G) and a copy of the radiology and/or operative reports from the tissue removal procedure *and* the diagnostic anatomic pathology report must be sent with the tissues to the ETCTN Biorepository.

For all archival tissue, the corresponding anatomical clinical pathology report is required both in the package and uploaded in the ETCTN specimen tracking system. If this is not available at the time of shipment, then it must be sent to the ETCTN Biorepository as soon as possible and uploaded to the ETCTN specimen tracking system, or the specimen will not be processed. The pathology report must state the disease diagnosis made by the reviewing pathologist.

5.6.2 Specimen Shipping Instructions

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

Frozen specimens and archival (FFPE) tissue may be shipped on Monday through Thursday.

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

5.6.2.1 Shipping of FFPE Blocks and Glass Slides

1. Before packaging blocks or slides, verify that each specimen is labeled according to Section 5.4.2.2.
2. Blocks should be placed in a hard-sided container, preferably a special block holder, to protect the specimen. Glass slides are to be placed in plastic slide holders. Place tissue paper on top of the separated slides prior to closing the slide holder to reduce slide movement during shipment.
3. Place the blocks or slides in a reinforced cardboard shipping box with appropriate packaging filler to minimize movement of specimens within the shipping box.
4. Include a copy of the forms listed above and a shipping manifest from the Specimen Tracking System with each shipment.
5. Please include a cold pack when shipping on hot days and extra insulation on cold days.
6. Ship specimens to the address listed below. FedEx Priority Overnight is strongly recommended to prevent delays in package receipt.

5.6.2.2 Shipping Frozen and Ambient Specimens in a Dual-Chamber Kit

The Dual Chambered Specimen Procurement Kit is constructed to allow the shipment of frozen (on dry ice) and ambient (room temperature) specimens in the same container. **Dry ice may be placed in either compartment of the kit but should not be put in both.** The dual chambered kit is only used for shipments that contain both frozen and ambient specimens. If formalin-fixed tissue is shipped separately (not in the same shipment as frozen specimens), then it must be shipped using institutional shipping supplies.

1. Before packaging specimens, verify that each specimen is labeled according to the instructions in 5.4.2 and that lids of all primary receptacles containing liquid are tightly sealed.
2. Pre-fill one of the kit chambers about 1/3 with dry ice.
3. Prepare the frozen specimens for shipment:
    a. Place the specimens into zip-lock bags.
    b. Place the zip-lock bags into a biohazard envelope containing absorbent material. Expel as much air as possible before sealing the biohazard envelope.
    c. Put each biohazard envelope into a Tyvek envelope. Expel as much air as possible and then seal the Tyvek envelope.
4. Quickly place the Tyvek envelope containing frozen specimens in the kit compartment that is pre-filled with dry ice. Place the Tyvek envelope on top of the dry ice. Cover the specimens with additional dry ice until the compartment is almost completely full.
5. Place the Styrofoam lid on top to secure specimens during shipment. Do not tape the inner chamber shut.
6. Prepare the ambient specimens for shipment:
    a. Seal the lids of the formalin jars with parafilm. Place absorbent material around the primary container of each liquid specimen. Place the specimens into zip-lock bags.
    b. Place specimens inside the secondary pressure vessel with bubble wrap.
    c. Secure the lid on the secondary pressure vessel and set it inside the kit chamber.
7. Insert a copy of the required forms in the kit chamber with the ambient specimens.
8. Place the Styrofoam lid on top of the kit compartment to secure specimens during shipment. Do not tape the inner chamber shut.
9. Close the outer lid of the Specimen Procurement Kit and tape it shut with durable sealing tape. Do not completely seal the container.
10. Complete a FedEx air bill and attach to top of shipping container.
11. Complete a dry ice label.
12. Attach the dry ice label and an Exempt Human Specimen sticker to the side of the shipping container.
13. Ship specimens via overnight courier to the address below. FedEx Priority Overnight is strongly recommended to prevent delays in package receipt.

5.6.3 Shipping Address

Ship to the address below. Ship 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. There is no central Courier account for this study. Sites are responsible for all costs for shipments to the ETCTN Biorepository.

5.6.4 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

5.7 Biomarker Plan

List of Biomarker Assays in Order of Priority

Priority	Biomarker Name	Assay (CLIA: Y/N)	Use in the Trial and Purpose	Specimens Tested	Collection Time Points	Mandatory or Optional	Assay Laboratory and Lab PI
Tissue-based Biomarkers							
1	Whole Exome Sequencing	NGS CLIA: N	Integrated To identify additional predictive biomarkers of response to AZD1775 (e.g. *TP53,* DNA repair mutations including ATM, ATR, ARX or other mutations).	DNA from Esophageal cancer biopsy (FFPE)	Archival (FFPE) or baseline	M	NCLN Genomics Laboratory Mickey Williams, Ph.D. mickey.williams@nih.gov
2	ɣH2AX, pNBS1 IFA with βCATN segmentation	Multiplex IF CLIA: N	Integrated To assess target engagement and response	Esophageal cancer biopsy (Frozen tissue)	For expansion cohort only: Baseline Week -1 Day 4 or 5	M	NCLN PD Assay Laboratory at MD Anderson Kate Ferry-Galow, Ph.D. ferrygalowkv@mail.nih.gov
3	p-HH3 (S20), p-cdk1 (Y15), pWEE1(S642), and p-Chk1(S345) IHC	IHC CLIA: N	Exploratory To assess target engagement and treatment response	Esophageal cancer biopsy (FFPE)	For expansion cohort only: Baseline Week -1 Day 4 or 5	M	City of Hope Terence Williams terwilliams@coh.org
Blood-based Biomarkers							

Priority	Biomarker Name	Assay (CLIA: Y/N)	Use in the Trial and Purpose	Specimens Tested	Collection Time Points	Mandatory or Optional	Assay Laboratory and Lab PI
1	Whole Exome Sequencing	NGS CLIA: N	Integrated To identify additional predictive biomarkers of response to AZD1775 (e.g. *TP53,* DNA repair mutations including ATM, ATR, ARX or other mutations).	DNA from Peripheral blood	Baseline (within 28 days prior to study entry)	M	NCLN Genomics Laboratory Mickey Williams, Ph.D. mickey.williams@nih.gov

5.8 Integrated Correlative Studies

5.8.1 Whole Exome Sequencing
5.8.1.1 Specimens Receipt and Processing at the ETCTN Biorepository

FFPE tissue blocks will be sectioned to generate an initial hematoxylin and eosin (H&E)-stained slide. All H&E stained slides will undergo a pathology QA review and annotation for macrodissection. Following macrodissection, tumor tissue from unstained slides will be scraped for co-extraction of DNA and RNA. The nucleic acids will be analyzed to determine concentration and quality. Aliquots of DNA and RNA will be shipped to the central sequencing laboratory for analysis.

DNA will be extracted from blood collected in EDTA tubes at baseline. Blood DNA will be quantitated, and then stored in a -80°C freezer until shipping for analysis.

5.8.1.2 Site Performing Correlative Study

Whole exome sequencing will be performed at the NCLN Genomics Laboratory under the supervision of Dr. Mickey Williams (mickey.williams@nih.gov)

5.8.1.3 Contact information for notification of specimen shipment

Dr. Mickey Williams
mickey.williams@nih.gov
5.8.2 γH2AX, pNBS1 IFA with βCATN segmentation
5.8.2.1 Specimens Receipt and Processing at the ETCTN Biorepository

Upon receipt at the Biorepository, the snap-frozen tissue will be accessioned, barcoded, and banked in a liquid nitrogen vapor phase freezer until distribution for testing.

5.8.2.2 Site Performing Correlative Study

γH2AX, pNBS1 IFA with βCATN segmentation will be performed at the NLCN PD Laboratory under the supervision of Dr. Kate Ferry-Galow (ferrygalowkv@mail.nih.gov).

5.8.2.3 Contact information for notification of specimen shipment

Dr. Kate Ferry-Galow
ferrygalowkv@mail.nih.gov

5.9 Exploratory/Ancillary Correlative Studies

5.9.1 p-HH3 (S20), p-cdk1 (Y15), pWEE1(S642), and p-Chk1(S345) IHC
5.9.1.1 Specimens Receipt and Processing at the ETCTN Biorepository

Tissue received in formalin will be processed and embedded in paraffin, and stored as an FFPE

tissue block. Samples should be removed from formalin and placed in 70% ethanol or proceed to paraffin embedding 24-36 hours after collection. FFPE blocks can be stored at room temperature. FFPE tissue blocks should be sent directly from the biorepository to the Williams Laboratory.

5.9.1.2 Site Performing Correlative Study

p-HH3 (S20), p-cdk1 (Y15), pWEE1(S642), and p-Chk1(S345) immunohistochemistry will be performed by the Williams Laboratory at City of Hope under the supervision of Dr. Terence Williams (terwilliams@coh.org).

5.9.1.3 Contact information for notification of specimen shipment

Samples should be shipped to the following address for analysis:

Terence Williams, M.D., Ph.D. Lab
Lipman Graff Building B202, City of Hope
1500 E Duarte Rd.
Duarte, CA 91010
Cell Phone (614) 477-6086
Lab phones (626) 218-4357 ext. 8-4357
Email: terwilliams@coh.org

Shipments should be made Monday through Wednesday only.

- Please notify Dr. Williams by e-mail on or before the day of submission with (1) the name of the contact person, (2) when to expect the sample, and (3) the overnight shipping carrier and tracking number of the package.

6. TREATMENT PLAN

6.1 Agent Administration

Treatment will be administered on an inpatient or 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.

Dose Escalation Schedule		
Dose Level	**Dose**	
	AZD1775 (mg)	**Radiation Therapy (Gy)**
Level -1	150 mg QD on Days 1 and 5 of weeks -1, 1, and 3	37.5 Gy/15 fractions

Level 1*	150 mg QD on Days 1, 3, and 5 of weeks -1, 1, and 3	37.5 Gy/15 fractions
Level 2	200 mg QD on Days 1, 3, and 5 of weeks -1, 1, and 3	37.5 Gy/15 fractions
Level 3	200 mg QD on Days 1-5 of weeks -1, 1, and 3	37.5 Gy/15 fractions
** Starting dose*		

Regimen Description					
Agent	***Premedications; Precautions***	***Dose***	***Route***	***Schedule***	***Cycle Length***
AZD1775	• 5-HT3 antagonist, ondansetron (Zofran) 8 mg PO QD or granisetron (Kytril) 1 mg PO QD prior to each AZD1775 dose* • Dexamethasone 4mg PO**	***	PO 2-3 hours **before** radiation therapy in weeks 1 and 3	As specified in dose escalation table	21 days (3 weeks)
Radiation Therapy	N/A	37.5 Gy in 15 fractions over 3 weeks	N/A	QD on Days 1-5, weeks 1-3	
**May be administered by IV as needed.* ***At a minimum, administer with AZD1775 on the first day of each 3-5 day dosing period, unless contraindicated or not well-tolerated.* ****Doses as appropriate for assigned dose level.*					

All patients will be required to have a 3-week washout period prior to starting systemic therapy. If previously on systemic therapy, a 3-week washout period is required prior to initiating study therapy.

The first week of treatment will consist of AZD1775 monotherapy (week -1). AZD1775 will then be administered orally daily 2-3 hours prior to radiation therapy according to the dose level schedule during weeks 1 and 3. Patients will receive AZD1775 concurrently with radiation. Radiation therapy will be delivered daily, five days per week, for a total dose of 37.5 Gy in 15 fractions during weeks 1-3.

The dose escalation component will be followed by an expansion cohort consisting of 12 patients to confirm safety with mandatory repeat EGD (esophagogastroduodenoscopy) on week -1 during

AZD1775 monotherapy to verify target engagement through pharmacodynamic studies. Of note, assessment of biopsy tissue for target engagement will not be conducted in real time or be used to inform recruitment of additional patients in the expansion cohort. During week -1, patients will be treated with AZD1775 at the MTD determined during dose escalation. On Days 4 or 5 of week -1, the patients will undergo repeat EGD with tumor biopsy (pharmacodynamic or PD biopsy) prior to receiving radiation therapy. On week 1, patients will continue AZD1775 while receiving radiation therapy. On week 2, patients will only receive radiation treatment. On week 3, patients will resume treatment with AZD1775 in addition to receiving radiation treatment. Patients on the expansion cohort will also be assessed for changes in dysphagia using the Ogilvie dysphagia score, a validated instrument for assessing dysphagia caused by esophageal cancer (a 5 graded scale where 0 is normal diet with no dysphagia and 4 is inability to swallow anything) (see Appendix E).

The patient will be requested to maintain a medication diary (Appendix C) of each dose of medication. The medication diary will be returned to clinic staff at the end of each course. Non-compliance based on assessment of the medication diary will result in removal from the study as documented in 6.5.

6.1.1 CTEP IND Agent

For patients on QD dosing, all patients must receive a 5-HT3 antagonist, ondansetron (Zofran) 8 mg PO QD or granisetron (Kytril) 1 mg PO QD prior to each dose of AZD1775. If nausea and vomiting continue, a second dose of antiemetics can be taken 8 hours later if necessary. In addition, dexamethasone 4 mg PO will be given with each AZD1775 dose, as a minimum on the first day of dosing AZD1775 of every 3-5 days dosing period, unless contraindicated or not well-tolerated. Dexamethasone or the 5-HT3 antagonist may be given by IV route as needed.

Promethazine (Phenergan), prochlorperazine (Compazine), and benzodiazepine may still be used as additional adjunctive treatments during AZD1775 therapy.

Suitable alternative medications may be used, with adequate justification, in those studies where the use of any of the above medications might interfere with other study procedures or are deemed insufficient.

Aprepitant and fosaprepitant are not permitted due to known drug interactions with AZD1775. Nausea and vomiting will be considered refractory if it does not resolve to ≤ Grade 1 with treatment with a combination of at least 2 of the antiemetics within 24 hours. Patients should be strongly encouraged to maintain liberal oral fluid intake.

6.1.2 Radiation Therapy

Radiation therapy will be administered in 15 QD fractions 5 days per week. The prescription dose per fraction is 2.5 Gy. The total prescription dose is 37.5 Gy. Radiation therapy is scheduled on trial for 5 days each week of the study, generally Monday through Friday of each week.

6.1.2.1 Immobilization and Simulation

Immobilization
Proper immobilization is critical for this protocol. Patient setup reproducibility must be achieved using appropriate clinical devices. Patients should be positioned supine in an individualized immobilization device on the CT table.

Simulation Imaging
Motion assessment during simulation is not required, but may be performed on patients to account for the tumor and nodal excursion with respiration and the appropriate type of treatment planning CT required.

A motion management technique-specific treatment planning CT (e.g., 4DCT, breath-hold, gating, Active Breathing Control (ABC) etc.) can be performed during simulation to define gross tumor volume (GTV*)*, clinical target volume (CTV), and planning target volume (PTV) (see definitions in Section 6.1.2.2). Slice thickness should be a maximum of 3-5 mm in slice thickness for regions including gross disease and 8-10 mm for other areas. A uniform 5 mm thickness is also acceptable. The field of view must be large enough so that none of the patient's anatomy along the path of the treatment beams is excluded.

A treatment planning FDG-PET/CT scan (or FDG-PET alone) (if available for simulation) with the patient in the treatment position is encouraged for treatment planning, but not required. In the case where the CT is obtained in the treatment position as part of radiation simulation, the CT from this study may be used as the planning CT scan. CT based treatment planning should include, superiorly, the entire thorax, and, inferiorly, below the kidneys. In preparation for the planning CT scan, patients will be asked to fast for 2 hours prior to simulation and treatment to minimize the volume of stomach contents.

Intravenous and esophageal contrast during the planning CT are optional at the discretion of the treating physician. If contrast is used, the densities or RLSPs should be over-ridden or the contrast scan must be registered to a non-contrast scan for planning purposes.

6.1.2.2 Definition of Target Volumes and Margins

Detailed Specifications
Target volumes: The definitions of volumes will be in accordance with the 1999 ICRUReport#62.

GTVp_3750, IGTVp_3750: The GTVp includes the GTV defined as the primary tumor in the esophagus. GTVp should be contoured on the primary CT dataset. If 4DCT simulation is performed for nongated free-breathing delivery, the phases of the breath cycle should be used to construct the composite GTV volume (IGTVp). Ideally, targets should be contoured on all phase images in order to construct IGTV. Some commercial software like MIM may be used to populate the physician's GTV contour on one-phase images to the other phase images. If such software is unavailable, at least 4 phase images (end of inspiration, mid-inspiration, end of

expiration and mid-expiration) should be used to assure IGTV accuracy. Maximum Intensity Projected (MIP) images should not be used to generate IGTV (Chen *et al*., 2014).

GTVn_3750, IGTVn_3750: The GTVn is defined as any grossly involved regional lymph nodes, either considered suspicious on FDG-PET/CT or proven by EUS biopsy, which is included at the discretion of the treating radiation oncologist. GTVn should be contoured on the primary CT dataset. If 4DCT simulation is performed, the phases of the breath cycle should be used to construct the composite GTV volume (IGTVn). Ideally, targets should be contoured on all phase images in order to construct IGTVn. Some commercial software like MIM may be used to populate the physician's GTV contour on one phase images to the other phase images. If such software is unavailable, at least 4 phase images (end of inspiration, mid-inspiration, end of expiration and mid-expiration) should be used to assure IGTVn accuracy. Maximum Intensity Projected (MIP) images should not be used to generate IGTVn (Chen *et al*., 2014).

GTV_3750, IGTV_3750: structure combined to include GTVp_3750 and GTVn_3750 for GTV_3750 or IGTV_3750 which will include IGTVp_3750 plus IGTVn_3750 if 4DCT simulation is performed.

CTV_3750: The CTV is defined as the GTV_3750 or IGTV_3750 with a 0.5 cm uniform expansion and edited back as needed for structures where there is unlikely to be invasion. Elective nodal radiation should not be performed.

PTV_3750: Additional margin shall be added to the CTV for set up error and movement. This expansion should be 0.5 to 1.0 cm and does not need to be uniform in all dimensions.

6.1.2.3 Definition of Critical Structures and Margins

Contouring and dose assessment of the following normal structures is encouraged.

Standard Name	Description
SpineCanal	Spinal Canal
Lungs	Right + Left Lung
Lung_R	Right Lung
Lung_L	Left Lung
Heart	Heart
Liver	Liver
Kidney_R Kidney_L	Right Kidney Left Kidney
Bowel_Small	Small bowel
Bowel_Large	Large bowel
Stomach	Stomach

Detailed Specifications

SpineCanal: Boundaries: Cranial: 1st slice of CT; Caudal: last slice of CT;

Lungs: Boundaries: Cranial: From apex bilaterally; Caudal: to bottom of L2
Heart: Boundaries: Base: Bottom of the aortic arch; Inferior: Apical most of the ventricle
Liver: Boundaries: Cranial: From dome; Caudal: Inferior tip
Kidneys: Entire kidneys contoured separately as Kidney_R and Kidney_L
Bowel_Small: Boundaries: Level of PTV
Bowel_Large: Boundaries: Level of PTV
Stomach: Entire stomach including cardia, fundus, body, antrum, and pylorus.

6.1.2.4 Dose Prescription

The PTV_3750 should receive a dose of 37.5 Gy in 15 fractions. This table should be used as a guide for treatment planning purposes.

Structures	Goal
Heart	V42 <10 cc
Bowel_Small (including duodenum)	V39 <05 cc
Bowel_Large	V45 <10 cc
Stomach	V39 <50 cc
Liver	V30 <700 cc
Kidney_R, Kidney_L	V37.5 <15 cc
Lungs	V16 <950 cc
SpineCanal	V39 <05 cc
Target Volume	Goal
PTV_3750	D1cc ≤ 110% V100% ≥ 95%

6.1.2.5 Treatment Technology

Photon therapy is required using either 3D conformal or intensity modulated radiation therapy (IMRT). IMRT may be delivered using multiple fixed fields employing dynamic multi-leaf collimator, helical arc therapy, or volumetric modulated arc therapy. Proton therapy is not permitted.

6.1.2.6 Online treatment image guided localization and treatment

Image-guided radiation should be performed daily and can be accomplished with either 2-dimensional kilovoltage (KV) images or 3-dimensional cone beam imaging at the discretion of the treating radiation oncologist.

6.2 Definition of Dose-Limiting Toxicity

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

The DLT observation window will include the entire duration of AZD1775 monotherapy and radiation followed by a 3-week break/DLT evaluation period. DLTs will be graded in severity according to CTCAE v5.0. A DLT will be defined as an adverse event that is related (possibly, probably, or definitely) to administration of AZD1775 and fulfills one of the following criteria:

- grade 4 neutropenia for ≥5 days or resulting in hospitalization for treatment of neutropenic fever
- grade 3 or 4 thrombocytopenia for ≥4 days
- grade ≥3 diarrhea if it is refractory to treatment as outlined in Section 6.4.4.1, and unable to be corrected to Grade 2 or less within 24 hours. Bloody or Grade 4 diarrhea will be dose-limiting.
- grade 4 esophagitis
- Any other grade 3 or 4 nonhematologic toxicity owing to treatment (with the exception of alopecia, nausea, and vomiting) requiring therapy to be held for >1 week, or recurrent or unacceptable grade 3 or 4 toxicity due to the treatment. Grade 3 nausea and vomiting will only be considered dose-limiting if it is refractory to anti-emetic therapy and unable to be corrected to Grade 1 or less within 24 hours.
- Rise in creatinine to Grade 3, not corrected to Grade 1 or less within 48 hours with IV fluids will be considered dose-limiting. All Grade 4 rises in creatinine will be dose limiting.
- Grade ≥ 3 metabolic toxicities unable to be corrected to Grade 1 or baseline within 48 hours (hypocalcemia or hypercalcemia, hypomagnesemia or hypermagnesemia, and hyponatremia) will be considered dose limiting. For hypokalemia or hyperkalemia, grade ≥ 2 toxicities unable to be corrected to grade 1 or less within 48 hours will be considered dose limiting. Grade 4 metabolic toxicities that are symptomatic will be considered dose-limiting regardless of duration or ability to correct.
- Failure to tolerate 90% of the dosing (both radiation therapy and AZD1775) and failure to remain on the study for 21 days due to toxicity will be considered a DLT.
- Dose-limiting hematologic and non-hematologic toxicities will be defined differently and will be based on events that occur during study drug administration and for 3 weeks after the completion of radiation therapy (total of six weeks).

We will employ the Bayesian optimal interval (BOIN) design to find the MTD (Liu and Yuan, 2015; Yuan *et al*., 2016) (see Section 9.1). Dose-limiting toxicity (DLT) is defined above.

For the BOIN design, the target toxicity rate for the MTD is 25%. Patients will be enrolled and treated in cohorts of size 2 starting at dose level 1. The BOIN design does not require a fixed cohort size and allows for decision making at any time during the trial by comparing the observed DLT rate at the current dose with the escalation and de-escalation boundaries. Therefore, the subsequent cohort size could be subject to change depending on the DLT data observed (but will not exceed 4).

6.3 Dose Expansion Cohorts:

Once the MTD is reached, an additional 12 patients will be treated at this dose. For the expansion cohort, patients will continue to be monitored for occurrence of DLT. We will use the elimination boundaries in Table 2 for toxicity monitoring. If 3 of the first 5 patients or if ≥4 of 6 patients experience DLT, the Principal Investigator will discuss with all study investigators and with CTEP whether further addition of patients is needed to re-assess the MTD. Monitoring of all safety and toxicity data is done by the Principal Investigator and the Corresponding Organization on a real-time basis as data are entered into Medidata Rave using the Web

Reporting Module. All participating sites are expected to notify the Principal Investigator when a DLT has occurred.

6.4 General Concomitant Medication and Supportive Care Guidelines

Because there is a potential for interaction of AZD1775 with other concomitantly administered drugs, the case report form must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies. The Principal Investigator should be alerted if the patient is taking any agent known to affect or with the potential for drug interactions. The study team should check a frequently-updated medical reference for a list of drugs to avoid or minimize use of. Appendix D (Patient Drug Interactions Handout and Wallet Card) should be provided to patients.

6.4.1 Interactions with CYP proteins

AZD1775 is a weak reversible inhibitor of CYP3A4 (IC_{50} 14 mcM). A formal clinical drug interaction study (D6014C00006) evaluating the effect of AZD1775 on PK of substrates for CYP3A, CYP2C19, and CYP1A2 has been conducted in advanced solid tumor patients. The results of this study suggest that AZD1775 is a weak inhibitor of these enzymes and is unlikely to result in clinically significant drug interaction.

In vitro studies suggest that the major pathways of the metabolism of AZD1775 in humans involve CYP3A4. Potent or moderate inhibitors or inducers of CYP3A4, sensitive CYP3A4 substrates, and CYP3A4 substrates with a narrow therapeutic window should be avoided until additional data on drug-drug interaction become available.

In vitro data suggests that AZD1775 may be a weak reversible inhibitor of CYP2C19 (IC_{50} 12 mcM). Caution should therefore be exercised when AZD1775 is co-administered with agents that are sensitive substrates of CYP2C19, or substrates of this enzyme with a narrow therapeutic range.

6.4.2 Interactions with protein transporters

Transporter studies (*in vitro*) have shown that AZD1775 is both a substrate and inhibitor (IC_{50} 20 mcM) of P-glycoprotein (P-gp). Maximum impact of these finding is likely to occur for drugs administered orally at the same time as AZD1775. Caution should therefore be exercised when agents that are inhibitors or substrates of P-gp are administered concomitantly with AZD1775.

Recent *in vitro* transporter studies have shown AZD1775 to be an inhibitor of breast cancer resistance protein (BCRP) (IC_{50} 5.1 mcM). This finding is particularly relevant for drugs administered orally where exposure is normally limited by BCRP-mediated efflux, in particular some statins such as rosuvastatin. Other drugs where the disposition is mediated via BCRP should be administered with caution, dose modification should be considered, or the drug should be substituted by an alternative drug.

AZD1775 has been shown to be an inhibitor of multidrug and toxin extrusion protein 1

(MATE1) (IC_{50} 0.132 mcM) and multidrug and toxin extrusion protein 2 (MATE2K) transporters (IC_{50} 0.307 mcM). A drug interaction with substrates of either transporter cannot be ruled out, the most important substrate known to date being metformin.

6.4.3 Drug-drug interactions

An exploratory assessment of the effect of aprepitant on AZD1775 exposure in oncology patients suggests that there is a drug interaction between AZD1775 and aprepitant, as exposure to AZD1775 increased by ~40% when aprepitant was co-administered with AZD1775. The observed increase in AZD1775 exposure is likely the result of CYP3A4 inhibition by aprepitant. This increase in exposure is statistically significant. At the selected MTDs, this increase may also be of clinical importance. Therefore, it is recommended that AZD1775 should not be co-administered with aprepitant (or a prodrug of aprepitant) until further evaluation.

The administration of AZD1775 with substances known to prolong the QTc interval is not recommended. Special note: the potential risk of concomitant use of AZD1775 with ondansetron (known to prolong the QTc interval) should be taken into account.

Potent or moderate inhibitors or inducers of CYP3A4, sensitive CYP3A4 substrates, and CYP3A4 substrates with a narrow therapeutic window should be avoided until additional data on drug-drug interactions (DDI) becomes available. The use of sensitive substrates of CYP3A4, such as atorvastatin, simvastatin and lovastatin, is prohibited in this study. As grapefruit and Seville oranges are known to contain moderate inhibitors of CYP3A4, these fruits or their products (including marmalade, juice, etc.) should be avoided while taking AZD1775.

6.4.4 AE Management guidelines

6.4.4.1 Diarrhea

If diarrhea develops and does not have an identifiable cause other than study drug administration or radiation therapy, anti-diarrheals such as Lomotil (diphenoxylate HCl 2.5 mg + atropine sulfate 0.025 mg/tablet) dosed according to package insert or loperamide 4 mg po after the first unformed stool with 2 mg po with every 2 hours as long as unformed stools continue (4 mg every 4 hours while asleep). No more than 16 mg of loperamide should be taken during a 24-hour period. This regimen can be repeated for each diarrheal episode. Diarrhea will be considered refractory if it does not resolve within 24 hours ≤ to Grade 2 with the above medications. If the patient develops blood or mucus in the stool, dehydration, or hemodynamic instability, or fever along with the diarrhea, anti-diarrheals will be discontinued and the patient will be treated with IV fluids and antibiotics as medically indicated.

6.4.5 Neutropenia

To reduce the risk of severe myelosuppression events, a complete blood count (CBC) should be performed weekly. Febrile neutropenia is a life-threatening complication requiring hospitalization and urgent broad spectrum antibiotics, as well as an aggressive search for the source and microbial cause of the episode. Growth factors to prevent neutropenia will not be

administered prophylactically. If necessary, they may be administered according to accepted American Society of Clinical Oncology (ASCO) guidelines to allow re-treatment.

6.4.6 Anemia

Symptomatic anemia should be treated with red blood cell transfusion and is recommended if the hemoglobin falls below 8 g/dL. The initiation of erythropoietic therapy for the management of chemotherapy-induced anemia follows the American Society of Hematology/ASCO clinical practice guidelines (http://www.asco.org).

6.4.7 Thrombocytopenia

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

6.4.8 Cardiac disorders

AZD1775 partially reversibly inhibited the hERG current with an IC_{50} value of 6.9 mcM in whole-cell voltage clamp studies. Tachycardia, and QTc prolongation have been observed in clinical studies with AZD1775, either as monotherapy or in combination with cytotoxic chemotherapy.

Attention should be paid to any detected increases in QTc interval (See Section 7.2).

6.4.9 Gastrointestinal hemorrhage

Gastrointestinal hemorrhage is a potential risk for which a causal association with AZD1775 has not yet been established. As of 11 November 2019, AstraZeneca has received five SAEs of gastrointestinal hemorrhagic events originating from AstraZeneca sponsored studies in AZD1775 monotherapy (Investigator's Brochure 2020).

Hematemesis and/or melaena are clear indicators of gastrointestinal hemorrhage. Localization and control of the bleeding should be the primary method of medical intervention. When required, transfusions may be indicated. Where investigations for sub-clinical or occult gastrointestinal hemorrhage prove negative, other causes of refractory anemia should be considered and investigated.

6.4.10 Infections and Infestations (Sepsis)

Sepsis has been added as an important potential risk for AZD1775. As part of the normal sepsis surveillance of oncology patients on known myelosuppressive treatments, Investigators should also be aware of the possibility of sepsis arising in association with the use of AZD1775 and be prepared to reduce dosing of AZD1775 or withdraw it where a causal relationship is suspected.

Sepsis can be so severe that it could lead to death. Patient discussions and patient's decisions must be documented about: 1) whether the physician has determined that therapy is beneficial and, if so, 2) whether or not the patient has chosen to continue the treatment.

6.4.11 Nausea and vomiting (Emesis)

All patients on QD dosing will receive anti-emetics as described in Section 6.1.1.

6.5 Duration of Therapy

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

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

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

6.6 Duration of Follow-Up

Patients will be examined 3 weeks after completing treatment, then followed every 3 months by a doctor visit for the first 2 years after completion of therapy, after removal from study, or until death, whichever occurs first. Patients will then be followed every 6 months for 3 years by doctor visit. Patients removed from study for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event.

7. DOSING DELAYS/DOSE MODIFICATIONS

Dose Level	AZD1775 Dose
-1	150 mg QD on Days 1 and 5 of weeks -1, 1, and 3
1*	150 mg QD on Days 1, 3, and 5 of weeks -1, 1, and 3
2	200 mg QD on Days 1, 3, and 5 of weeks -1, 1, and 3
3	200 mg QD on Days 1-5 of weeks -1, 1, and 3
* *Starting dose*	

Patients will be allowed up to two dose reductions.

Dose adjustments will be based on the organ system exhibiting the greatest degree of toxicity. Dose reductions or holds and initiation of supportive care are allowed as clinically indicated by the treating physician.

If further dose modification is considered necessary, a discussion must take place with the Medical Monitor and the Medical Monitor needs to provide approval for the patient to continue.

Any patient requiring a toxicity-related dose delay of more than 21 days from the intended day of the next scheduled dose must be discontinued from the study unless there is approval from the Medical Monitor for the patient to continue.

7.1 Dose modifications due to hematologic toxicity for adavosertib monotherapy

Complete blood counts (CBC) will be obtained for all patients **at the beginning of each treatment week and reviewed prior to dose administration.** If hematologic toxicity occurs, treatment should be held, and ANC and platelets should be monitored at least weekly. Patients should be managed as medically indicated. Treatment should not be resumed **until recovery to Grade 1** (ANC $\geq$1.5 x 10^9/L or 1,500/ mcL; platelets $\geq$75,000/ mcL).

Table 1. Neutrophil and Platelet Blood Counts and Study Drug Action

Neutrophil count	**Action Adavosertib**	**2nd event**	**Action Adavosertib**	**3rd event**	**Action Adavosertib**
Grade 2 <1.5-1.0 x10^9/L	Investigator judgement to continue adavosertib or interrupt until Grade 1 (≥1.5-1.0 x 109/L)				
Grade 3 <1.0-0.5 x10^9/L	Hold Resume at same dose		Hold Resume at DL-1		Hold Resume at DL-2
Grade 3 <1.0-0.5 x10^9/L with documented infection and/or fever	Hold Resume at DL-1		Hold Resume at DL-2		Hold Contact Medical Monitor
Grade 4 <0.5 x10^9/L	Hold Resume at DL-1		Hold Resume at DL-2		Discontinue and follow for disease progression
Grade 4 Febrile neutropenia or Grade 4 Infection with neutropenia	Discontinue and follow for disease progression				
Platelet count	**Action Adavosertib**	**2nd event**	**Action Adavosertib**	**3rd event**	**Action Adavosertib**
Grade 2 <75,000-50,000/μL	Hold Resume at same dose		Hold Resume at DL-1		Hold Resume at DL-1

Table 1. Neutrophil and Platelet Blood Counts and Study Drug Action

Grade 3 <50,000- 25,000/ µL	Hold Resume at DL-1	Hold Resume at DL-2	Hold Resume at DL-2
Grade 4 <25,000/ µL without any evidence of bleeding	Hold Resume at DL-1	Hold Resume at DL-2	Discontinue and follow for disease progression
Thrombocytopenic haemorrhage (gross occult bleeding) associated with platelet count <50,000/ µL	Discontinue and follow for disease progression		

DL-1 = One dose level lower; DL-2 = Two dose levels lower

Please consider using G-CSF in the event of severe neutropenia or febrile neutropenia according to institutional standards.

No more than two dose reductions will be allowed for any patient. Patients requiring further dose reduction due to toxicity must discontinue study treatment. Dose re-escalation is not allowed. If the patient has concurrent neutropenia and thrombocytopenia, please follow the most conservative guidance in the table below and discuss with Medical Monitor as needed.

If hematological parameters do not recover within 21 days, the patient should be removed from the study treatment.

Neutropenia, infection, febrile neutropenia dose modifications and management

Any day	
Grade 3 neutropenic fever (ANC <1000/μL + Temperature ≥101°F [38.5°C]) or neutropenic infection **Documented infection with Grade 3 neutropenia** (ANC <1000/μL) **Grade 4 neutropenia** (ANC <500/μL >7 days **Grade 4 thrombocytopenia** (platelet count <25,000/μL >7 days)	**Hold dose until recovery.** **Then, upon resuming dosing, reduce AZD1775[a].**
Grade 4 febrile neutropenia or Grade 4 infection with neutropenia (both defined as septic shock) **Thrombocytopenic haemorrhage** (gross occult bleeding) associated with a platelet count <50,000/μL	**Discontinue treatment and follow for disease progression.**

[a] No more than two dose reduction will be allowed for any patient. Patients requiring an additional dose modification to AZD1775 due to toxicity will discontinue study treatment.

7.2 Non-hematologic toxicity dose modifications

Substantial acute toxicities should be managed as medically indicated and with temporary suspension of investigational product, as appropriate. Dose reductions or holds and initiation of supportive care are allowed as clinically indicated by the treating physician. Dose reductions of AZD1775 should be considered if the toxicity is considered to be related to AZD1775, i.e. in monotherapy studies or in combination studies if relationship cannot be wholly attributed to the combination agent (each combination agent should be considered on an individual basis). Dose re-escalation is not permitted. In general, if a patient experiences a G1/G2 non-hematological toxicity, no dose modification is required. If a patient experiences a G3 or G4 toxicity which is not attributable to the disease or disease-related processes under investigation, dosing will be interrupted and/or the dose reduced, and supportive therapy administered as required. Any patient who develops a Grade 3 or 4 non-hematologic toxicity that does not resolve to ≤ Grade 1 within 21 days should be removed from the study treatment unless approved by the Medical Monitor.

Table 2: QTc interval prolongation (AZD1775 dose modifications)

QTc Value (triplicate)[a, b]	**Action to be taken**

QTc 481-500 ms (Grade 2) [c]	Hold treatment for up to 21 days. Consider seeking cardiologist's advice. Once QTc interval has returned to Grade 1 and correction of possible electrolyte imbalance has been made, treatment may resume at the same dose level.
QTc ≥ 501 ms or Shift from baseline of ≥ 60ms (Grade 3)[c]	Hold treatment for up to 21 days. Seek cardiologist's advice and correct any electrolyte imbalance. - If QTc interval recovers to ≤ 480 ms (Grade 1) or baseline if there was a shift from baseline of ≥ 60ms, then may resume treatment at next lower dose level, if cardiologist agrees. - If QTc interval doesn't recover to ≤ 480 ms or baseline if there was a shift from baseline of ≥ 60ms, then discontinue treatment permanently
Torsade de pointes or polymorphic ventricular tachycardia or signs/symptoms of serious arrhythmia (Grade 4)[c]	Discontinue treatment permanently. Seek cardiologist's advice.

[a] Mean triplicate value
[b] Based on a dedicated QT study conducted to evaluate the effect of single & multiple doses of AZD1775 225 mg on QT prolongation in advanced solid tumor patients, there was no significant relationship found between ΔQTcF and AZD1775 concentration. Model prediction of change in QTcF at the geometric mean of C_{max} on Day 1 (712.8 nM) was -2.405 msec (90% CI: -5.939, 1.129). Similarly, model prediction of QTcF at the geometric mean C_{max} on Day 3 (1462 nM) was -0.7568 msec (90% CI: -5.148, 3.634). The upper limit of the predicted 90% CIs for ΔQTcF were well below 10 msec, supporting the conclusion that AZD1775 does not have a clinically relevant impact on cardiac repolarization.
[c] Per CTCAE V 5.0
ECG should be assessed if electrolyte abnormalities are detected or suspected (e.g. in relation to gastrointestinal toxicity). If a prolongation of the QTc interval is detected this must be closely monitored as clinically indicated.

7.3 Dose modifications to radiation therapy are defined below:

- Radiation therapy will typically continue as prescribed without modifications while drug-related adverse events are being managed.

- Radiation therapy will be interrupted for grade >3 non-hematologic toxicity at least possibly related to radiation therapy. If the patient develops > grade 3 toxicity at least possibly related to radiation therapy, radiation therapy and AZD1775 should be withheld. Interruption of therapy may continue until the toxicity has regressed to grade ≤2 to allow resumption of therapy; however, every effort should be made to limit treatment

interruptions to 1-2 weeks.

- Management of radiation-related toxicity is left to the discretion of the treating radiation oncologist.

- Missed radiation treatments should ideally be made up if reasonable to do so, such that the total dose delivered is 37.5 Gy in 15 fractions. Ultimate discretion to complete the radiation course is left to the treating physician team.

- In the unlikely event that radiation must be permanently discontinued, patients will be taken off-study.

Patients experiencing grade >2 toxicity during treatment are required to visit the study doctor after completion of treatment every other week until toxicity resolves to grade ≤2.
Definition of Complete Course:

A cycle is defined as 21 days. If a patient receives 90% of scheduled treatments of AZD1775 and radiation therapy and remains in the study until D21, the patient will be considered to have completed the therapy. Patients who do not complete the therapy for reasons other than toxicity will be replaced. Patients who do not complete the therapy due to toxicity will not be replaced. All patients should have completed therapy (unless removed from the study due to toxicity) to be evaluable.

8. PHARMACEUTICAL INFORMATION

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

8.1 AZD1775 (NSC 751084)

Chemical Name: 2-allyl-1-[6-(1-hydroxy-1-methyl-ethyl)-2-pyridyl]-6-[4-(4-methylpiperazin-1-yl)anilino]pyrazolo[3,4-d]pyrimidin-3-one hemihydrate

Other Names: MK-1775, adavosertib

Classification*:* inhibitor of WEE1 kinase

CAS: 1277170-60-1

Molecular Formula: $C_{27}H_{32}N_8O_2 \cdot 0.5H_2O$ **M.W.:** 500.6

Mode of Action: AZD1775 inhibits WEE1 which phosphorylates and inhibits cyclin-dependent kinases 1 (CDK1) and 2 (CDK2), and is involved in regulation of the intra-S and G2 cell cycle checkpoints. In in vitro and in vivo preclinical models, AZD1775 selectively enhanced chemotherapy induced death of cells deficient in p53 signaling.

Description: AZD1775 is a crystalline, hemihydrate form of the drug substance.

How Supplied: AZD1775 is supplied by AstraZeneca and distributed by the Pharmaceutical Management Branch, CTEP/DCTD/NCI as capsules available in 25 mg (yellow color, size 2 gelatin capsule) and 100 mg (orange color, size 2 gelatin capsule) strengths. The dry-filled capsules consist of a roller-compacted granule of drug substance, lactose monohydrate, microcrystalline cellulose, croscarmellose sodium, and magnesium stearate. Each high density polypropylene (HDPE) bottle contains 20 capsules.

Storage: Store at 2 to 30˚C (36 to 86˚F). Do not freeze.

If a storage temperature excursion is identified, promptly return AZD1775 to between 2-30°C and quarantine the supplies. Provide a detailed report of the excursion (including documentation of temperature monitoring and duration of the excursion) to PMBAfterHours@mail.nih.gov for determination of suitability.

Stability: Shelf-life stability studies of AZD1775 capsules are ongoing.

Route of Administration: Oral administration. Capsules should not be opened.

Potential Drug Interactions: AZD1775 is primarily metabolized by CYP3A4 and is a weak, time-dependent inhibitor of CYP3A4. Avoid concomitant CYP3A4 moderate or strong inhibitors/inducers, and sensitive substrates with a narrow therapeutic index. AZD1775 is also a weak inhibitor of CYP2C19. Caution should be exercised with concomitant administration of sensitive substrates or substrates with a narrow therapeutic index.

In vitro transporter studies have shown that AZD1775 was an inhibitor of OATP1B1, OATP1B3, MATE1, MATE2K, P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP), and a substrate for P-gp and BCRP. The PK parameters of AZD1775 could be altered if AZD1775 is coadministered with P-gp and BCRP inhibitors/inducers, and there is potential for drug-drug interactions when coadministered with OATP1B1, OATP1B3, MATE1, MATE2K, P-gp and BCRP substrates. This finding is particularly relevant for drugs administered orally where exposure is normally limited by BCRP-mediated efflux, in particular some statins. Modelling has predicted a substantial increase in the exposure of atorvastatin when coadministered with AZD1775 and the use of atorvastatin is therefore prohibited.

Availability

AZD1775 is an investigational agent supplied to investigators by the Division of Cancer Treatment and Diagnosis (DCTD), NCI. AZD1775 is provided to the NCI under a Collaborative Agreement between the Pharmaceutical Collaborator and the DCTD, NCI (see Section 13.5).

8.1.1 Agent Ordering and Agent Accountability

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

In general, sites may order initial agent supplies when a subject is being screened for enrollment onto the study.
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.

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

8.1.2 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.

8.1.3 Useful Links and Contacts

- CTEP Forms, Templates, Documents: http://ctep.cancer.gov/forms/
- NCI CTEP Investigator Registration: RCRHelpDesk@nih.gov
- PMB policies and guidelines: http://ctep.cancer.gov/branches/pmb/agent_management.htm
- PMB Online Agent Order Processing (OAOP) application: https://ctepcore.nci.nih.gov/OAOP
- CTEP Identity and Access Management (IAM) account: https://ctepcore.nci.nih.gov/iam/
- CTEP IAM account help: ctepreghelp@ctep.nci.nih.gov
- IB Coordinator: IBCoordinator@mail.nih.gov

- PMB email: PMBAfterHours@mail.nih.gov
- PMB phone and hours of service: (240) 276-6575 Monday through Friday between 8:30 am and 4:30 pm (ET)

9. STATISTICAL CONSIDERATIONS

9.1 Study Design/Endpoints

This is a phase 1 study to evaluate the safety of adding a WEE1 kinase inhibitor, AZD1775, to palliative radiation therapy followed by a 12-patient expansion cohort to verify safety and target engagement at the MTD. The phase 1 portion of the study will employ a BOIN design where the target toxicity rate for the MTD is 25% with 75% dose-elimination cut-off and the maximum sample size is 18 DLT evaluable patients. Patients will be enrolled and treated in cohorts of size 2 starting at dose level 1 with the study design described as follows:

1. The first cohort of 2 patients is treated at dose level 1.
2. A dose escalation/de-escalation will be conducted according to the rule displayed in **Table 3** below to assign a dose to the next cohort of patients (subsequent cohort size may be subject to change, but will not exceed 4). First find the column for the total number of patients treated at the current dose in **Table 3** below:
    a. If the cumulated total # of DLTs at the current dose is less than or equal to n1 in the column of the corresponding total number of patients treated at the current dose, there will be dose escalation.
    b. If the cumulated total # of DLTs at the current dose is greater than or equal to n2 in the column of the corresponding total number of patients treated at the current dose, there will be dose de-escalation.
    c. If the cumulated total number of DLTs at the current dose is greater than or equal to n3, the current and higher doses will be eliminated. "Eliminate" means that the current and higher doses will be eliminated from the trial to prevent treating any future patients at these doses because they are overly toxic.
    d. When a dose is eliminated, there will automatically be de-escalation from the dose to the next lower level. When the lowest dose is eliminated, the trial will be stopped for safety. In this case, no dose should be selected as the MTD.
    e. If none of the actions (*i.e.*, escalation, de-escalation or elimination) is triggered, new patients will be treated at the current dose.
    f. If the current dose is the lowest dose (dose level -1) and the rule indicates dose de-escalation, new patients will be treated at the lowest dose unless the number of DLTs reaches the elimination boundary, at which point the trial will be terminated for safety.
    g. If the current dose is the highest dose and the rule indicates dose escalation, new patients will be treated at the highest dose.

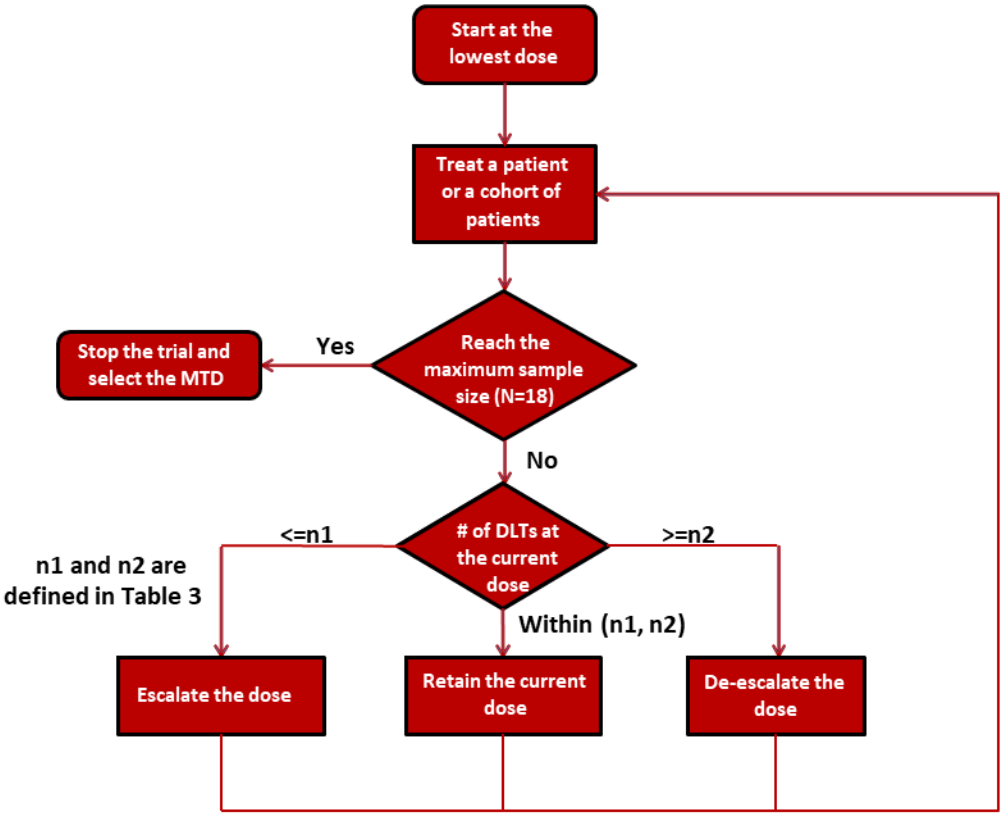
3. Repeat step 2 until the maximum sample size of 18 DLT-evaluable patients is reached or stop the trial if the number of patients treated at the current dose reaches 10.

After the study is completed, the MTD will be selected based on isotonic regression, as specified. Specifically, the MTD will be selected as the dose for which the isotonic estimate of the toxicity rate is closest to the target toxicity rate (*i.e*. 25%). If there are ties, the higher dose level will be selected when the isotonic estimate is lower than the target toxicity rate; and the lower dose level will be selected when the isotonic estimate is greater than the target toxicity rate. Of note, the protocol team can choose, for safety reasons and clinical judgment, to not escalate doses when permitted by design and select a dose lower than the MTD as the recommended phase 2 dose.

Table 3: Dose escalation/de-escalation rule for the BOIN design.

Actions	The number of patients treated **at the current dose**									
	1	**2**	3	**4**	5	**6**	7	**8**	9	**10**
Escalate if # of DLT ≤	0	**0**	0	**0**	0	**1**	1	**1**	1	**1**
De-escalate if # of DLT ≥	1	**1**	1	**2**	2	**2**	3	**3**	3	**3**
Eliminate if # of DLT ≥	NA	**NA**	2	**2**	2	**2**	3	**3**	3	**4**

BOIN Design Flowchart



Once we determine the MTD, an additional 12 patients (expansion cohort) will be enrolled for additional experience with safety and efficacy. We will use the elimination boundaries in Table 3 for toxicity monitoring.

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

Operating characteristics

Table 4 shows the operating characteristics of the trial design based on 10,000 simulations of the trial using the BOIN Design Desktop Program (Venier *et al*., 2018). The operating characteristics show that the design selects the true MTD, if any, with high probability and allocates more patients to the dose levels with the DLT rate closest to the target of 0.25.

Table 4. Operating Characteristics of the BOIN design.

	Dose Level				Number of Patients	% Early Stopping
	-1	1	2	3		
Scenario 1						
True DLT Rate	0.25	0.42	0.48	0.53		
Selection %	40.7	9.8	2.4	0.5		46.6
# Pts Treated	6.6	3.3	1.0	0.3	11.15	
Scenario 2						
True DLT Rate	0.01	0.10	0.25	0.40		
Selection %	8.9	40.7	37.9	12.3		0.2
# Pts Treated	3.0	5.6	5.6	3.1	17.28	
Scenario 3						
True DLT Rate	0.02	0.06	0.10	0.25		
Selection %	3.0	9.2	41.3	46.1		0.4
# Pts Treated	2.6	3.3	5.2	6.2	17.23	
Scenario 4						
True DLT Rate	0.05	0.07	0.10	0.12		
Selection %	3.7	7.5	14.3	72.4		2.1
# Pts Treated	2.9	3.2	3.5	7.1	16.63	

9.2 Sample Size/Accrual Rate

The maximum number of patients included in this study will be 18 for the phase 1 portion and 12 for the expansion cohort with an additional three patients recruited for ~10% attrition. Planned accrual is one patient per month.

PLANNED ENROLLMENT REPORT

DOMESTIC PLANNED ENROLLMENT REPORT (TREATMENT)					
Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/ Alaska Native	0	0	0	0	0
Asian	1	2	0	0	3
Native Hawaiian or Other Pacific Islander	1	2	0	0	3
Black or African American	2	2	0	0	4
White	4	19	0	0	23
More Than One Race	0	0	0	0	0
Total	8	25	0	0	33

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9.3 Analysis of Secondary Endpoints

Symptom relief rate will be calculated with 95% binomial confidence intervals (assuming the number of patients is binomially distributed). Time to second intervention for dysphagia will be defined as the time from initiation of therapy to the time of second intervention for dysphagia. Overall survival will be defined as the duration from patient enrollment to death due to any cause. Patients last known to be alive will censored at the date of last contact. Survival will initially be analyzed using Kaplan-Meier methods, resulting in median survival times with 95% CI. Ogilvie dysphagia scores will be summarized at baseline and post-treatment, and the improvement will be compared using paired t-test or Wilcoxon signed-rank test between baseline and post-treatment. Biomarkers will be described graphically or summary measures (*e.g.* mean and standard errors, or median and range) and compared between responders and non-responders using a two sample t-test or Wilcoxon test if the data is not normally distributed.

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 List (CAEPR)

10.1.1 CAEPR for CTEP IND Agent: AZD1775 (adavosertib, NSC 751084)

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 323 patients.* Below is the CAEPR for AZD1775 (adavosertib).

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

Version 2.7, April 27, 2020[1]

Adverse Events with Possible Relationship to AZD1775 (adavosertib) (CTCAE 5.0 Term) [n= 323]			**Specific Protocol Exceptions to Expedited Reporting**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
	Anemia		***Anemia (Gr 3)***
		Febrile neutropenia	
CARDIAC DISORDERS			
		Atrial fibrillation	
		Supraventricular tachycardia	
GASTROINTESTINAL DISORDERS			
	Abdominal pain		***Abdominal pain (Gr 2)***
	Constipation		***Constipation (Gr 2)***
Diarrhea			***Diarrhea (Gr 3)***
	Dyspepsia		
		Gastrointestinal hemorrhage[2]	
	Mucositis oral		***Mucositis oral (Gr 2)***
Nausea			***Nausea (Gr 3)***
Vomiting			***Vomiting (Gr 3)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Edema limbs		***Edema limbs (Gr 2)***
Fatigue			***Fatigue (Gr 3)***
	Fever		***Fever (Gr 2)***
HEPATOBILIARY DISORDERS			
		Hepatobiliary disorders - Other (hepatitis)	
INFECTIONS AND INFESTATIONS			
	Infection[3]		***Infection[3] (Gr 3)***
INVESTIGATIONS			
	Alanine aminotransferase increased		***Alanine aminotransferase increased (Gr 3)***
		Electrocardiogram QT corrected interval prolonged	
	Lymphocyte count decreased		
	Neutrophil count decreased		***Neutrophil count decreased (Gr 4)***

Adverse Events with Possible Relationship to AZD1775 (adavosertib) (CTCAE 5.0 Term) [n= 323]			Specific Protocol Exceptions to Expedited Reporting
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
	Platelet count decreased		***Platelet count decreased (Gr 4)***
	Weight loss		
	White blood cell decreased		***White blood cell decreased (Gr 4)***
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		***Anorexia (Gr 2)***
	Dehydration		
	Hypokalemia		***Hypokalemia (Gr 2)***
	Hypomagnesemia		***Hypomagnesemia (Gr 2)***
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Back pain		***Back pain (Gr 2)***
	Muscle cramp		
	Myalgia		***Myalgia (Gr 2)***
NERVOUS SYSTEM DISORDERS			
`	Dizziness		***Dizziness (Gr 2)***
	Headache		***Headache (Gr 2)***
		Intracranial hemorrhage	
PSYCHIATRIC DISORDERS			
	Insomnia		
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Cough		***Cough (Gr 2)***
	Dyspnea		***Dyspnea (Gr 2)***
		Hypoxia	
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Rash[4]		***Rash[4] (Gr 2)***
VASCULAR DISORDERS			
		Phlebitis	

[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.

[2]Gastrointestinal hemorrhage includes Anal hemorrhage, Cecal hemorrhage, Colonic hemorrhage, Duodenal hemorrhage, Esophageal hemorrhage, Esophageal varices hemorrhage, Gastric hemorrhage, Hemorrhoidal hemorrhage, Ileal hemorrhage, Intra-abdominal hemorrhage, Jejunal hemorrhage, Lower gastrointestinal hemorrhage, Oral hemorrhage, Pancreatic hemorrhage, Rectal hemorrhage, Retroperitoneal hemorrhage, and Upper gastrointestinal hemorrhage under the GASTROINTESTINAL DISORDERS SOC.

[3]Infection includes all 75 sites of infection under the INFECTIONS AND INFESTATIONS SOC.

[4]Rash may include rash, erythema, eczema, and rash maculo-papular.

[5]Peripheral neuropathy includes both peripheral motor neuropathy and peripheral sensory neuropathy.

[6]Acute kidney injury includes renal impairment and acute renal insufficiency.

Adverse events reported on AZD1775 (adavosertib) trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that AZD1775 (adavosertib) caused the adverse event:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Blood and lymphatic system disorders - Other (pancytopenia); Blood and lymphatic system disorders - Other (thrombocytosis); Blood and lymphatic system disorders - Other (right leg deep vein thrombosis); Leukocytosis
Cardiac disorders - Cardiac disorders - Other (cardiomegaly); Chest pain - cardiac; Myocardial infarction; Palpitations; Sinus bradycardia; Sinus tachycardia
EAR AND LABYRINTH DISORDERS - Ear pain; Hearing impaired; Tinnitus
EYE DISORDERS - Blurred vision; Cataract; Eye disorders - Other (eye swelling); Eye pain; Keratitis; Photophobia; Scleral disorder; Vision decreased; Watering eyes
GASTROINTESTINAL DISORDERS - Abdominal distension; Anal pain; Ascites; Belching; Bloating; Cheilitis; Colitis; Colonic obstruction; Dry mouth; Duodenal ulcer; Dysphagia; Enterocolitis; Flatulence; Gastric ulcer; Gastritis; Hemorrhoids; Oral pain; Rectal pain; Small intestinal obstruction
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Chills; Death NOS; Edema trunk; Flu like symptoms; Gait disturbance; General disorders and administration site conditions - Other (catheter site pain); Infusion site extravasation; Malaise; Non-cardiac chest pain; Pain
IMMUNE SYSTEM DISORDERS - Allergic reaction; Anaphylaxis; Cytokine release syndrome
INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Fall; Injury, poisoning and procedural complications - Other (excoriation); Injury, poisoning and procedural complications - Other (ligament sprain)
INVESTIGATIONS - Alkaline phosphatase increased; Aspartate aminotransferase increased; Blood bilirubin increased; Creatinine increased; GGT increased; Investigations - Other (blood urea increased); Lymphocyte count increased
METABOLISM AND NUTRITION DISORDERS - Alkalosis; Hypercalcemia; Hyperglycemia; Hyperkalemia; Hyperuricemia; Hypoalbuminemia; Hypocalcemia; Hyponatremia; Hypophosphatemia; Tumor lysis syndrome
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthralgia; Arthritis; Bone pain; Flank pain; Generalized muscle weakness; Muscle weakness lower limb; Musculoskeletal and connective tissue disorder - Other (groin pain); Neck pain; Pain in extremity
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS) - Neoplasms benign, malignant and unspecified (incl cysts and polyps) - Other (carcinoid tumor); Tumor pain
NERVOUS SYSTEM DISORDERS - Central nervous system necrosis; Cognitive disturbance; Depressed level of consciousness; Dysesthesia; Dysgeusia; Encephalopathy; Lethargy; Nervous system disorders - Other (hemiparesis); Paresthesia; Peripheral neuropathy[5]; Presyncope; Somnolence; Syncope
PSYCHIATRIC DISORDERS - Agitation; Anxiety; Confusion; Depression
RENAL AND URINARY DISORDERS - Acute kidney injury[6]; Hematuria; Urinary frequency; Urinary incontinence; Urinary retention; Urinary tract pain
REPRODUCTIVE SYSTEM AND BREAST DISORDERS - Genital edema; Reproductive system and breast disorders - Other (female genital tract fistula)
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Allergic rhinitis; Apnea; Bronchopulmonary hemorrhage; Epistaxis; Hiccups; Nasal congestion; Pleural effusion; Pneumonitis; Pulmonary hypertension; Respiratory, thoracic and mediastinal disorders - Other (diaphragmalgia); Voice alteration; Wheezing
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Alopecia; Bullous dermatitis; Dry skin; Hyperhidrosis; Pain of skin; Palmar-plantar erythrodysesthesia syndrome; Pruritus; Purpura; Rash acneiform; Skin ulceration; Urticaria
VASCULAR DISORDERS - Flushing; Hematoma; Hot flashes; Hypertension; Hypotension; Thromboembolic event

Note: AZD1775 (adavosertib) 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.

10.2 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 website http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

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

- **Attribution** of the AE:
  - Definite – The AE *is clearly related* to the study treatment.
  - Probable – The AE *is likely related* to the study treatment.
  - Possible – The AE *may be related* to the study treatment.
  - Unlikely – The AE *is doubtfully related* to the study treatment.
  - Unrelated – The AE *is clearly NOT related* to the study treatment.

10.3 Expedited Adverse Event Reporting

10.3.1 Rave-CTEP-AERS Integration

The Rave Cancer Therapy Evaluation Program Adverse Event Reporting System (CTEP-AERS) integration enables evaluation of post-baseline AEs entered in Rave to determine whether they require expedited reporting, and facilitates entry in CTEP-AERS for those AEs requiring expedited reporting.

All AEs that occur after baseline are collected in Medidata Rave using the Adverse Event form, which is available for entry at each treatment or reporting period, and used to collect AEs that start during the period or persist from the previous reporting period. The CRA will enter AEs that occur prior to the start of treatment on a baseline form that is not included in the Rave-CTEP-AERS integration. AEs that occur prior to enrollment must begin and end on the baseline Adverse Event form and should not be included on the standard Adverse Events form that is available at treatment unless there has been an increase in grade.

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

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

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

Upon completion of AE entry in Medidata Rave, the CRA submits the AE for rules evaluation by completing the Expedited Reporting Evaluation form. Both NCI and protocol-specific reporting rules evaluate the AEs submitted for expedited reporting. A report is initiated in CTEP-AERS using information entered in Medidata Rave for AEs that meet reporting requirements. The CRA completes the report by accessing CTEP-AERS via a direct link on the Medidata Rave Expedited Reporting Evaluation form.

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

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

- Study specific documents: Protocols > Documents > Education and Promotion, and
- Expedited Safety Reporting Rules Evaluation user guide: Resources > CTSU Operations Information > User Guides & Help Topics.

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

- NCI Guidelines for Investigators: Adverse Event Reporting Requirements is available at https://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf.

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 as long as the death occurred within 30 days after the last administration of the investigational agent. Attribution to treatment or other cause must be provided.

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

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

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

10.4 Routine Adverse Event Reporting

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

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

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.

11. STUDY CALENDAR

Baseline evaluations are to be conducted within 2 weeks prior to start of protocol therapy. Scans must be done ≤4 weeks prior to the start of therapy. In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

	Pre-Treatment			During Treatment					Follow-Up	
	≤12 months prior to study entry	≤28 days prior to study entry	≤14 days prior to study entry	Wk -1	Wk 1	Wk 2	Wk 3	Wk 6	Q3 months (+/- 1 month) for first 2 years	Q6 months (+/- 1 month) for years 2-5
AZD1775[a]				X	X		X			
Informed consent		X								
History and physical exam			X		X	X	X	X	X	X
Vital signs			X		X	X	X	X	X	X
Weight			X		X	X	X	X	X	X
Performance status[c]			X		X	X	X	X	X	X
CBC w/diff, plts			X		X	X	X	X	X	X
Comprehensive Chemistry Panel[d]			X		X	X	X	X	X	X
EKG		X	As clinically indicated							
Pregnancy test[e]		X		X[f]						
Adverse event evaluation[g]				X----------X						
CT chest, abdomen, and pelvis or CT chest, MRI abdomen and pelvis, or whole-body PET-CT		X								
EGD with biopsy	X		X[b]	X[b,h]						

	Pre-Treatment			During Treatment					Follow-Up	
	≤12 months prior to study entry	≤28 days prior to study entry	≤14 days prior to study entry	Wk -1	Wk 1	Wk 2	Wk 3	Wk 6	Q3 months (+/- 1 month) for first 2 years	Q6 months (+/- 1 month) for years 2-5
Radiation therapy[i]					X	X	X			
Ogilvie dysphagia score (expansion cohort)[j]			X					X	X	X
Whole Exome Sequencing (Formalin Fixed Biopsy)		X[k]								
Whole Exome Sequencing (blood in EDTA)		X								
ɣH2AX, pNBS1 IFA with βCATN segmentation (Snap Frozen Biopsy)			X[b]	X[b,h]						
p-HH3 (S20), p-cdk1 (Y15), pWEE1(S642), and p-Chk1(S345) IHC (Formalin Fixed Biopsy)			X[b]	X[b,h]						

	Pre-Treatment			During Treatment					Follow-Up	
	≤12 months prior to study entry	≤28 days prior to study entry	≤14 days prior to study entry	Wk -1	Wk 1	Wk 2	Wk 3	Wk 6	Q3 months (+/- 1 month) for first 2 years	Q6 months (+/- 1 month) for years 2-5

a: Dosing days as assigned. Dose should be taken 2-3 hours prior to radiation therapy in weeks 1 and 3.

b: Expansion cohort only.

c: Note: Performance status evaluations are based on a 4 week cycle. At minimum, performance status should be evaluated at the beginning of every cycle.

d: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus (as clinically indicated), potassium, total protein, SGOT [AST], SGPT [ALT], sodium.

e: Pregnancy test for women of childbearing potential.

f: To be performed on Day 1.

g: Patients with toxicity during treatment must visit the study doctor after completion of treatment every other week until toxicity resolves to grade ≤2.

h: To be performed on Day 4 or 5

i: Delivered five days per week, for a total dose of 37.5 Gy in 15 fractions.

j: See Appendix E for Ogilvie dysphagia scoring

k: If archival (FFPE) tissue is not available, a baseline biopsy is mandatory.

12. MEASUREMENT OF EFFECT

Although the clinical benefit of this drug has not yet been established, the intent of offering this treatment is to provide a possible therapeutic benefit, and thus the patient will be carefully monitored for tumor response and symptom relief in addition to safety and tolerability.

12.1 Antitumor Effect – Solid Tumors

12.1.1 Definitions

Evaluable for Toxicity. All patients will be evaluable for toxicity from the time of their first treatment with AZD1775.

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.)

12.1.2 Disease Parameters

Patient dysphagia will be evaluated using the Ogilvie dysphagia score, comparing pre-treatment to post-treatment scores (Appendix E).

12.1.3 Overall Survival

Overall survival will be defined as the duration from patient enrollment to death due to any cause. Patients last known to be alive will censored at the date of last contact. Survival will initially be analyzed using Kaplan-Meier methods, resulting in median survival times with 95% CI.

12.2 Other Response Parameters

12.2.1 Dysphagia

Time to second intervention for dysphagia will be defined as the time from initiation of therapy to the time of second intervention for dysphagia.

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.

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

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

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

Mandatory safety and trial review teleconferences will be scheduled and moderated by the Multi-Center Trial Program (MCTP). All sites involved in the study are expected to have a representative present for every call to review and discuss patients on study and other applicable agenda items. Meeting minutes will be used to document each teleconference. The minutes will be stored in the MCTP protocol files. Teleconferences must minimally be held monthly and may be held more frequently, as needed. For studies closed to accrual with patients expected to remain on long-term treatment and/or follow-up, teleconferences may be extended to occur every two months or quarterly. Decreasing frequency of teleconferences requires OSU PI and MCTP approval.

13.2 Data Reporting

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

Requirements to access Rave via iMedidata:

- A valid account, and
- Assigned a Rave role on the relevant LPO or PO roster at the enrolling site of: Rave CRA, Rave Read Only, Rave CRA (LabAdmin), Rave SLA, or Rave Investigator.
- Rave role requirements:

  - Rave CRA or Rave CRA (Lab Admin) role, must have a minimum of an Associate Plus (AP) registration type,
  - Rave Investigator role, must be registered as an Non-Physician Investigator (NPIVR) or Investigator (IVR), and
  - Rave Read Only role, must have at a minimum an Associates (A) registration type.
- Refer to https://ctep.cancer.gov/investigatorResources/default.htm for registration types and documentation required.

If the study has a DTL, individuals requiring write access to Rave must also be assigned the appropriate Rave tasks on the DTL.

Upon initial site registration approval for the study in Regulatory Support System (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 staff must log in to the Select Login (https://login.imedidata.com/selectlogin) using their CTEP-IAM username and password, and click on the *accept* link in the upper right-corner of the iMedidata page. Site staff will not be able to access the study in Rave until all required Medidata and study specific trainings are completed. Trainings will be in the form of electronic learnings (eLearnings), and can be accessed by clicking on the link in the upper right pane of the iMedidata screen. If an eLearning is required and has not yet been taken, the link to the eLearning will appear under the study name in iMedidata instead of the *Rave EDC* link; once the successful completion of the eLearning has been recorded, access to the study in Rave will be granted, and a *Rave EDC* link will display under the study name.

Site staff that have not previously activated their iMedidata/Rave account at the time of initial site registration approval for the study in RSS will receive a separate invitation from iMedidata to activate their account. Account activation instructions are located on the CTSU website in the Data Management section under the Rave resource materials (Medidata Account Activation and Study Invitation Acceptance). Additional information on iMedidata/Rave is available on the CTSU members' website in the Data Management > Rave section at www.ctsu.org/RAVE/ 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 three times annually (one annual site visit and two data audits). For CTMS monitored studies, after users have activated their accounts, please contact the Theradex Help Desk at (609) 619-7862 or by email at CTMSSupport@theradex.com for additional support with Rave and completion of CRFs.

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.

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 Data Quality Portal

The Data Quality Portal (DQP) provides a central location for site staff to manage

unanswered queries and form delinquencies, monitor data quality and timeliness, generate reports, and review metrics.

The DQP is located on the CTSU members' website under Data Management. The Rave Home section displays a table providing summary counts of Total Delinquencies and Total Queries. DQP Queries, DQP Delinquent Forms, and the DQP Reports modules are available to access details and reports of unanswered queries, delinquent forms, and timeliness reports. Review the DQP modules on a regular basis to manage specified queries and delinquent forms.

The DQP is accessible by site staff that are rostered to a site and have access to the CTSU website. Staff that have Rave study access can access the Rave study data using a direct link on the DQP.

To learn more about DQP use and access, click on the Help icon displayed on the Rave Home, DQP Queries, and DQP Delinquent Forms modules.

Note: Some Rave protocols may not have delinquent form details or reports specified on the DQP. A protocol must have the Calendar functionality implemented in Rave by the Lead Protocol Organization (LPO) for delinquent form details and reports to be available on the DQP. Site staff should contact the LPO Data Manager for their protocol regarding questions about Rave Calendaring functionality.

13.5 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.

13.6 Genomic Data Sharing Plan

The investigators and statistician and/or bioinformaticians for a study will have access to all data on mutations and variants stored in the Theradex Data Base. This information will be sequestered from access throughout the study until it is analyzed for purposes of reporting and publishing of the study results.

13.7 Incidental/Secondary Findings Disclosure Procedure

Incidental/secondary findings of germline and/or somatic mutations, confirmed by Sanger sequencing, will be relayed to the treating physician who is responsible for notifying the patients, with a genetic counselor present if that is deemed appropriate.

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APPENDIX A PERFORMANCE STATUS CRITERIA

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

APPENDIX B FORMULA TO ESTIMATE RENAL FUNCTION USING SERUM CREATININE

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

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

Formulae:

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

SCr in mg/dL; Output is in mL/min/1.73 m^2 and needs no further conversions.

2. eGFR using the Modification of Diet in Renal Disease (MDRD) Study (Levey *et al*., 2006).

$175 \times SCr^{-1.154} \times age^{-0.203} \times 0.742$ (if female) $\times$ 1.212 (if black)
Output is in mL/min/1.73 m^2 and needs no further conversions.

3. Estimated creatinine clearance (ClCr) by the Cockcroft-Gault (C-G) equation (Cockcroft and Gault, 1976).

$$CLcr\ (mL/min) = \frac{[140 - age\ (years)] \times weight\ (kg)}{72 \times serum\ creatinine\ (mg/dL)} \{\times 0.85\ for\ female\ patients\}$$

Followed by conversion to a value normalized to 1.73 m^2 with the patient's body surface area (BSA).

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APPENDIX C PATIENT MEDICATION DIARY

C.1 Medication Diary for AZD1775 - Days -7 through -1 (Week -1)

CTEP-assigned Protocol #__10389____
Local Protocol # _____

PATIENT'S MEDICATION DIARY – AZD1775

Today's date _____ **Agent** _____**AZD1775**_____

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

INSTRUCTIONS TO THE PATIENT:

1. Complete one form for each month.
2. Your doctor will tell you what days you should take AZD1775. You will take ___25 mg capsules and ___ 100 mg capsules on the designated treatment days. You should swallow the capsules whole. **Do not chew or open capsules**.
3. If you vomit shortly after you take your medications: the dose should only be replaced if all of the intact capsules can be seen and counted. Should 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 after that scheduled dose time. If greater than 2 hours after the scheduled dose time, do not take the missed dose. Take your assigned dose at the next scheduled time.
4. Record the date, the number of capsules of each size you took, and when you took them.
5. If you have any comments or notice any side effects, please record them in the Comments column.
6. Notify your doctor at the first sign of poorly formed or loose stools, or an increased frequency of bowel movements. Loperamide (Imodium) should be kept on hand and should be taken as recommended by your doctor.
7. Please return the forms to your physician when you go for your next appointment.

Day	Date	What time was dose taken?	# of capsules taken		Comments
			25 mg	100 mg	
1					
2					
3					
4					
5					
6					
7					

Physician's Office will complete this section:

1. Date patient started protocol treatment_____
2. Date patient was removed from study_____
3. Patient's planned total daily dose_____
4. Total number of capsules taken this month (each size)_____
5. Physician/Nurse/Data Manager's Signature _____

Patient's signature:_____

C.2 Medication Diary for AZD1775 - Cycle 1 (Weeks 1-3)

CTEP-assigned Protocol #__10389____
Local Protocol # _____

PATIENT'S MEDICATION DIARY – AZD1775

Today's date _____ **Agent** _____**AZD1775**_____

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

INSTRUCTIONS TO THE PATIENT:

1. Complete one form for each month.
2. Your doctor will tell you what days you should take AZD1775. You will take your dose of AZD1775 on designated days 2-3 hours prior to radiation therapy. You will take ___25 mg capsules and ___ 100 mg capsules on the designated treatment days. You should swallow the capsules whole. **Do not chew or open capsules**.
3. If you vomit shortly after you take your medications: the dose should only be replaced if all of the intact capsules can be seen and counted. Should 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 after that scheduled dose time. If greater than 2 hours after the scheduled dose time, do not take the missed dose. Take your assigned dose at the next scheduled time.
4. Shaded rows indicate the days that you **WILL NOT** take AZD1775.
5. Record the date, the number of capsules of each size 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. Notify your doctor at the first sign of poorly formed or loose stools, or an increased frequency of bowel movements. Loperamide (Imodium) should be kept on hand and should be taken as recommended by your doctor.
8. Please return the forms to your physician when you go for your next appointment.

Day	Date	What time was dose taken?	# of capsules taken		Comments
			25 mg	100 mg	
1					
2					
3					
4					
5					
6					
7					
8		X	X	X	X
9		X	X	X	X
10		X	X	X	X
11		X	X	X	X
12		X	X	X	X
13		X	X	X	X
14		X	X	X	X
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 capsules taken this month (each size)_____
5. Physician/Nurse/Data Manager's Signature _____

Patient's signature:_____

APPENDIX D PATIENT DRUG INTERACTIONS HANDOUT AND WALLET CARD

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

Patient Name:	**Diagnosis:**	**Trial #:**	10389
Study Doctor:	**Study Doctor Phone #:**	**Study Drug(s):**	AZD1775

Please show this paper to all your healthcare providers (doctors, physician assistants, nurse practitioners, pharmacists), and tell them you are taking part in a clinical trial sponsored by the National Cancer Institute.

AZD1775 (adavosertib) interacts with certain specific enzymes in the liver and certain transport proteins that help move drugs in and out of cells.

- The enzymes in question are ***CYP 3A4 and 2C19.*** AZD1775 (adavosertib) is metabolized by CYP3A4 and may be affected by other drugs that inhibit or induce these enzymes. AZD1775 (adavosertib) is an inhibitor of CYP 3A4 and 2C19 and may affect the metabolism of other drugs.
- The proteins in question are ***OATP1B1, OATP1B3, MATE1, MATE2K, P-gp, and BCRP.*** AZD1775 (adavosertib) is a substrate of P-gp and BCRP and may be affected by other drugs that inhibit or induce these transporters. AZD1775 (adavosertib) is an inhibitor of OATP1B1, OATP1B3, MATE1, MATE2K, P-gp, and BCRP and may affect transport of other drugs in and out of cells.

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

AZD1775 (adavosertib) 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 herbal supplements such as St. John's Wort. 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, or pharmacists) you are taking part in a clinical trial.

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

AZD1775 (adavosertib) must be used very carefully with other medicines that need certain **liver enzymes or transport proteins to be effective or to be cleared from your system.** 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, 2C19, OATP1B1, OATP1B3, MATE1, MATE2K, P-gp, and BCRP."**

- Please be very careful! Over-the-counter drugs (including herbal supplements) may contain ingredients that could interact with your study drug. Speak to your doctors or pharmacist to determine if there could be any side effects.
- 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

_____. May 2015

(Next page: Patient Drug Interaction Wallet Card)

PATIENT DRUG INTERACTION WALLET CARD

NIH NATIONAL CANCER INSTI	NIH NATIONAL CANCER INSTI	NIH NATIONAL CANCER INSTI	NIH NATIONAL CANCER INSTI
EMERGENCY INFORMATION		**DRUG INTERACTIONS**	
Show this card to all of your healthcare providers. Keep it with you in case you go to the emergency room.	Tell your doctors **before** you **start** or **stop** any medicines. **Check with your doctor or pharmacist if you need to use an over-the-counter medicine or herbal supplement!**	**Carry this card with you at all times** AZD1775 interacts with certain specific enzymes in the liver or other tissues like the gut and certain transport proteins that help move drugs in and out of cells and must be used very carefully with other medicines.	
Patient Name: **Diagnosis:** **Study Doctor:** **Study Doctor Phone #:** **NCI Trial #:** 10389 **Study Drug(S):** AZD1775 (adavosertib)	**Use caution and avoid the following drugs if possible:**	Your healthcare providers should be aware of any medicines that are strong inducers/inhibitors or substrates of CYP3A4, CYP2C19, OATP1B1, OATP1B3, MATE1, MATE2K, P-gp, and BCRP. **Before prescribing new medicines**, your health care provider should check a **frequently-updated medical reference** for a **list of drugs to avoid** or contact your study doctor.	Version 05/2015
For more information: 1-800-4-CANCER cancer.gov | clinicaltrials.gov	**For more information:** 1-800-4-CANCER cancer.gov | clinicaltrials.gov	**For more information:** 1-800-4-CANCER cancer.gov | clinicaltrials.gov	**For more information:** 1-800-4-CANCER cancer.gov | clinicaltrials.gov

Fold at dotted lines:

APPENDIX E OGILVIE DYSPHAGIA SCORING

Ogilvie's dysphagia score classification is as follows:

Grade	Description
0	Able to eat the ordinary diet
1	Able to eat solid food
2	Able to eat semisolids
3	Able to take liquids
4	Able to eat nothing

APPENDIX F PRE-BIOPSY ASSESSMENT

A pre-biopsy lesion assessment can increase trial safety and efficiency. By agreement between all investigators, an attempt at biopsy will be made if the clinical trial team determines that a biopsy poses minimal relative risk, provides potential clinical gain to the participant, and will likely yield sufficient tissue for analysis.

Pre-biopsy assessments will be reported and tracked through a trial-specific CRF within the CTEP Medidata Rave system. Additional information can be found in the Investigational Radiology SOP available at:
https://ctep.cancer.gov/initiativesPrograms/docs/ETCTN_IR_Research_Biopsy_SOP.pdf.

Individual Patient Pre-Biopsy Assessment. Gastroenterologist co-investigators are encouraged to apply this pre-biopsy scoring and correlation system to assist in the determination of biopsy appropriateness.

- Gastroenterologist co-investigators assign a subjective score of 1-3 based on likelihood of success due to lesion characteristics.

    1. Biopsy should not be done
        A. Due to safety concerns
        B. Due to lack of suitable lesion for biopsy

    2. Uncertainty about success
        A. Due to access path to lesion
        B. Due to lesion characteristics

    3. Likely successful

- Lesion characteristics to be considered
    - Size (small) (<2 cm)
    - Location/path to lesion
    - Morphologic features (necrosis, sub-solid, sclerosis, ill-defined/infiltrative)
    - PET (+/-), avidity
    - Organ/site (sclerotic bone is low yield; fine needle aspiration to be used)

APPENDIX G TISSUE BIOPSY VERIFICATION

A copy of the diagnostic pathology report must be shipped with all tissue specimens sent to the ETCTN Biorepository.

If the *corresponding* pathology report is not available for the biopsy, then a copy of the radiology report or operative report from the biopsy procedure and the diagnostic pathology report must be sent to the ETCTN Biorepository. A completed copy of this appendix (i.e., Tissue Biopsy Verification) must also be submitted to the ETCTN Biorepository.

Note: If this information is not provided with the biopsy specimen, then it will not be accepted by the ETCTN Biorepository.

Please have the Clinician* responsible for signing out this patient's case complete the following:

ETCTN Universal Patient ID: _____

ETCTN Patient Study ID: _____

Date of Procedure (mm/dd/yyyy): _____

Tissue Type (circle one): **Primary**

Time point (circle one): **Baseline** **Week -1 Day 4 or 5**

Site Tissue Taken From: _____

Diagnosis: _____

I agree that this tissue may be released for research purposes only and that the release of this tissue will not have any impact on the patient's care.

_____ _____
Clinician Signature Date

_____
Clinician Printed Name

*Note: For the purposes of this form, Clinician could include the Nurse Practitioner, Registered Nurse, Pathologist, Radiologist, Interventional Radiologist, Surgeon, Oncologist, Internist, or other medical professional responsible for the patient's care.

APPENDIX H NCLN PHARMACODYNAMICS LABORATORY FROZEN BIOPSY COLLECTION PROCEDURE

Title:	Tumor Frozen Needle Biopsy Specimen Collection and Handling				Page 1 of 11
Doc. #:	SOP340507	Revision:	G	Effective Date:	6/19/2019

Laboratory of Human Toxicology & Pharmacology

Applied/Developmental Research Directorate, Leidos Biomedical Research, Inc.

Frederick National Laboratory for Cancer Research

Technical Reviewer: Corey Evans Date: Corey J. Evans -S (Affiliate) Digitally signed by Corey J. Evans -S (Affiliate) Date: 2019.06.20 13:47:37 -04'00'

NCTVL Reviewer: Jiuping Ji Jiuping Ji -S (Affiliate) Digitally signed by Jiuping Ji -S (Affiliate) Date: 2019.07.05 10:34:23 -04'00'

IQC Approval: Katherine V. Ferry-Galow Date: Katherine V. Ferry-galow -S (Affiliate) Digitally signed by Katherine V. Ferry-galow -S (Affiliate) Date: 2019.07.22 14:46:16 -04'00'

LHTP Approval: Ralph E. Parchment Date: Ralph E. Parchment -S (Affiliate) Digitally signed by Ralph E. Parchment -S (Affiliate) Date: 2019.08.02 13:09:35 -04'00'

DCTD OD Approval: Toby Hecht Date: Toby T. Hecht -S Digitally signed by Toby T. Hecht -S Date: 2019.08.02 16:29:51 -04'00'

Change History

Revision	Approval Date	Description	Originator	Approval
G	6/19/2019	Minor updates to collection and shipping procedures.	KFG	REP
F	2/11/2015	Updated contact shipping address and process for advance notification of shipments.	KFG	REP
E	7/3/2013	Updated tube-type to 1.5-mL conical bottom screw cap tubes to allow for broader use in DCTD assays and minimize the need to transfer biopsies during sample extraction steps. Decreased maximum time from biopsy collection to freezing to 2 minutes.	YAE	REP
D	1/8/2013	Update handling in surgical suite including details on halving of biopsy. Record total time elapsed from biopsy collection to freezing.	YAE, MM	JJ
C	12/29/2010	Update sample snap freeze to dry ice/ethanol bath or liquid nitrogen.	YAE	JJ
B	7/24/2009	Updated SOP format and prepared for publication to the DCTD Biomarkers Web site	YAE	JJ
A	10/13/2006	Revision with New Shipping Address	YZ	JJ
--	8/25/2006	New Document	YZ	JJ

Please check for revision status of the SOP at

http://dctd.cancer.gov/ResearchResources/ResearchResources-biomarkers.htm

and be sure to use the current version.

leidos
Leidos Biomedical Research, Inc.

TABLE OF CONTENTS

1.0 PURPOSE

Standardize the method for collecting and handling frozen needle tumor biopsies to enable specimen use for measurement of pharmacodynamic (PD) markers following treatment with anticancer agents.

2.0 SCOPE

This procedure applies to all personnel involved in the collection and handling of frozen needle tumor biopsies for use in PD marker assays during clinical trials. The goal of this SOP and associated training is to ensure consistency in tumor needle biopsy collection and handling between clinical sites.

3.0 ABBREVIATIONS

DCTD	=	Division of Cancer Treatment and Diagnosis
ELISA	=	Enzyme-Linked ImmunoSorbent Assay
FNLCR	=	Frederick National Laboratory for Cancer Research
ID	=	Identification / Identifier
IQC	=	Internal Quality Control
LHTP	=	Laboratory of Human Toxicology and Pharmacology
NCTVL	=	National Clinical Target Validation Laboratory
PADIS	=	Pharmacodynamics Assay Development & Implementation Section
PD	=	Pharmacodynamic
SOP	=	Standard Operating Procedure

4.0 INTRODUCTION

Specimen handling, shipping, and storage procedures (pre-analytical variables) can have a significant impact on the reliability of biomarker measurements in the laboratory. Following detailed steps for sample collection and handling procedures and recording any deviations from this procedure allows retrospective identification of artifactual changes in biomarker readout and increases the reliability of the data and validity of the analytical results.

5.0 ROLES AND RESPONSIBILITIES

Laboratory Director/Supervisor	The Laboratory Director/Supervisor, directs laboratory operations, supervises technical personnel and reporting of findings, and is responsible for the proper performance of all laboratory procedures. Oversees the personnel who follow the SOPs in the laboratory and is responsible for ensuring the personnel are certified and have sufficient experience to handle clinical samples.
Certified Assay Operator and/or PK/PD Support Lab Personnel	A Certified Assay Operator and/or PK/PD Support Lab personnel may be a Laboratory Technician/Technologist, Research Associate, or Laboratory Scientist who has been certified through DCTD training on this SOP. Works under the guidance of the Laboratory Director/Supervisor. This person performs laboratory procedures and examinations in accordance with the current SOP(s), as well as any other procedures conducted by a laboratory, including maintaining equipment and records and performing quality assurance activities related to performance.

5.1 It is the responsibility of the Laboratory Director/Supervisor to ensure that all personnel have documented training and qualification on this SOP prior to the actual handling and processing of samples from clinical trial patients. The Laboratory Director/Supervisor is responsible for ensuring the Certified Assay Operator running the SOP has sufficient experience to handle and analyze clinical samples.

5.2 It is the responsibility of the Certified Assay Operator and/or PK/PD Support Lab personnel to confirm scheduled specimen collection time points, pre-print all labels and data collection sheets in advance, check documentation for accuracy, and verify that the required collection tubes, supplies, and equipment are available for successful collection and handling of biopsy samples.

5.3 The Certified Assay Operator and/or PK/PD Support Lab personnel responsible for conducting the specimen collection and handling procedures are to follow this SOP and complete the required tasks and associated documentation. The Batch Record (Appendix 1) must be completed in ***real-time*** for each experimental run, with each page ***dated and initialed*****,** and placed with the clinical sample information.

5.4 The responsible personnel are to check the DCTD Biomarkers Web site (http://dctd.cancer.gov/ResearchResources/ResearchResources-biomarkers.htm) to verify that the latest SOP version is being followed.

6.0 MATERIALS AND EQUIPMENT REQUIRED

6.1 Stopwatch, total time in minutes and seconds required
6.2 1.5-mL Sarstedt o-ring screw cap, conical bottomed tubes (Sarstedt, Cat#: 72.703.416)
6.3 Disposable, fine-tipped tweezers (e.g., VWR, Cat#: 83009-010). Tweezer tips need to easily fit to bottom of a 1.5-mL Sarstedt tube
6.4 Printable microcentrifuge tube labels or BSI labeling system
6.5 81-place freezer boxes (e.g., Fisher Scientific, Cat#: 12-565-182)
6.6 Thermoflask cooler or polystyrene foam container
6.7 Ice bucket
6.8 Liquid nitrogen or dry ice/ethanol bath
6.9 Wet ice
6.10 -80°C freezer (or lower)
6.11 Biohazard specimen bags
6.12 Insulating Styrofoam shipping container

7.0 OPERATING PROCEDURES

7.1 Record the name and certification number of the Certified Assay Operator and/or PK/PD Support Lab personnel performing the SOP, the facility/clinic collecting the specimens, the Patient/Sample ID, the primary diagnosis, and the clinical protocol number in the Batch Record (Appendix 1).

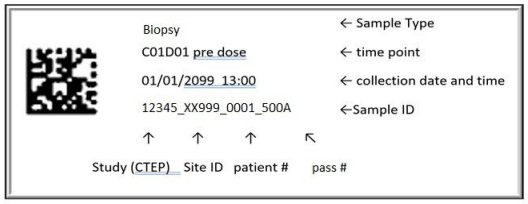
- The Batch Record for this SOP is sufficient for collection of a **single** set of biopsy samples collected from a single patient at a single timepoint. If collecting biopsy samples for more than one patient, prepare a separate Batch Record for each patient.

7.2 Labels

7.2.1 Prepare enough pre-printed specimen labels for each whole or halved biopsy sample to be collected and frozen as defined in the Pharmacodynamic/Correlative Study section of the Clinical Protocol; be sure to coordinate with the clinical center if they prepare the labels for sample collection. If two passes are collected from one tumor, the labels would be identical except that the specimen ID would be followed by a letter A/B to designate pass number. The specimen ID includes the CTEP protocol number followed by a unique patient identifier and a specimen series ID.

NCI tumor biopsy specimen IDs for PD sampling are series 500 with consecutive numbers identifying the collection time point as defined in the Clinical Protocol.

Sample pre-printed label for all frozen-tissue biopsy tubes:



7.2.2 Of the pre-printed labels prepared for each sample, one label will go on each 1.5-mL Sarstedt tube, one on the Batch Record (Appendix 1), and the last will be given to the research nurse to place into the patient record sheet. **Note:** be sure that no patient identifiable information is shown on the labels.

7.3 Tumor Needle Biopsy Collection and Handling

7.3.1 The research nurse is to notify the laboratory of scheduled PD sample collections, preferably giving at least 24 hours of notice. Arrive at the biopsy collection site early enough to allow sufficient time to set up laboratory supplies, collect relevant clinical information, and ensure rapid transport of specimens to the laboratory for placement at -80ºC (or lower) after collection.

7.3.2 Bring all necessary lab supplies including: disposable tweezers, a minimum of two 1.5-mL Sarstedt tubes (one for each whole biopsy core) pre-cooled on liquid nitrogen or dry ice/ethanol in an insulated bucket, and one pre-printed specimen label to give to the research nurse for the patient record.

Note: Pre-chill additional 1.5-mL Sarstedt tubes for specimen collection in case the interventional radiologist collects additional passes, or one of the other tubes is compromised prior to collection.

7.3.3 The total time elapsed between biopsy collection and placement into the pre-chilled tube is of **key importance** to biomarker analysis; biopsies should be frozen within **2 minutes** of collection. The interventional radiologist will eject the biopsy onto a sterile slide (for optimal analyte recovery the slide should be pre-chilled). Start a stopwatch (or note the time) at this point (Appendix 1, Section 1) and immediately walk the slide to the sample preparation table.

7.3.4 In the Batch Record (Appendix 1, Section 1), indicate if a full or halved biopsy, as defined in the Pharmacodynamic/Correlative Study section of the Clinical Protocol, is prepared.

7.3.4.1 For whole biopsies: Uncap an empty, pre-chilled 1.5-mL Sarstedt tube and, using disposable tweezers, pick up the freshly collected needle biopsy with the tweezers at one end, and touch the opposite end of the biopsy to the inner surface of the prechilled 1.5-mL Sarstedt tube. This should attach the tissue to the tube, allowing it to be dropped into the tube while releasing the tissue from the tweezers without sticking. Dispose of the tweezers in the appropriate biohazardous waste container(s).

7.3.4.2 For halved biopsies: Use 1-2 disposable tweezers and cut/shear the biopsy in half cross-wise while it is on the slide (do not pull or stretch the biopsy longitudinally). Use the tweezers to transfer the halved biopsies to sterile pre-chilled tubes as indicated above.

7.3.5 Immediately snap freeze the biopsy by placing the tube in liquid nitrogen or a dry ice/ethanol bath. **Note:** DO NOT let the tubes tip over in the dry ice/ethanol bath.

7.3.6 Calculate the total time elapsed from biopsy collection to biopsy freezing and record the total number of **minutes and seconds** elapsed in the Batch Record (Appendix 1, Section 1).

7.3.7 Note the specific location of each biopsy pass collected (*e.g.*, spleen, large left upper quadrant splenic mass) and a description of the appearance of the biopsy (*e.g.*, large whole core or small, fragmented core) in the Batch Record (Appendix 1, Section 1).

7.3.8 Note the biopsy timepoint (Appendix 1, Section 1).

7.4 If biopsy procedure details can be obtained from the interventional radiologist or research nurse, record them in the Batch Record (Appendix 1, Section 2). Some information may not be available until a later time from the clinical staff.

During **first-in-human** PD sample collection studies, information such as type of anesthesia and time-lag between biopsy needle withdrawal and sample freezing need to be tracked in order to determine the optimal sample collection procedure for the clinical community.

7.5 Return to the sample processing laboratory and transfer the frozen biopsy specimen(s) to -80°C (or lower) for storage until shipment to the PD processing laboratory. Record the date and time specimens are placed at -80ºC (or lower; Appendix 1, Section 3).

7.6 Review and finalize the Batch Record and document **ANY** and **ALL** deviations from this SOP in the Batch Record (Appendix 1, Section 5).

7.7 The Laboratory Director/Supervisor should review the Batch Record and sign to affirm the data contained within are correct (Appendix 1, Section 6).

8.0 SHIP TO FNLCR FOR ANALYSIS (OPTIONAL)

If shipping to a location other than FNLCR, use the following steps as a guide.

8.1 Sites are required to create a FedEx shipping label to accommodate the variable dry ice weight of the shipment package. Use only FedEx Priority Overnight Shipping. FNLCR PD Support will provide a FedEx account number to cover the cost of the shipment via NCI_PD_Support@mail.nih.gov.

8.1.1 By linking the FNLCR FedEx account number provided to the biopsy shipment, FNLCR PD Support can closely monitor the shipment and cover all shipping costs, and appropriate notification can be provided to pertinent staff of expected sample shipment arrival.

8.1.2 Please send all shipments via FedEx Priority Overnight shipping to the FNLCR PD Specimen Central Receiving address listed below:

Attention: Dan Danner
NCI-F/FNLCR
1073 Beasley Street, Building 1073
Fort Detrick
Frederick, MD 21701
Phone: (301) 846-5748

8.2 Once a tumor biopsy has been collected from a patient and placed at -80ºC (or lower), FNLCR PD Specimen Central Receiving should be notified that the specimens are ready for shipment. Preferably, if additional biopsies will be taken from the same patient (post-dose timepoint[s]), the biopsies are to be stored in a local freezer at -80ºC (or lower), and shipped together as a full set in one shipment.

8.3 Send an e-mail to FNLCR PD Specimen Central Receiving (NCI_PD_Support@mail.nih.gov) to advise that biopsy samples are being prepared for shipment. State "*Protocol Name* PD Specimens Ready for Shipment" in the subject line. Request a confirmation e-mail that personnel will be available on the expected delivery date and time. Personnel are generally available to receive frozen shipments Tuesday through Friday, exclusive of government holidays. If needed, FNLCR PD Central Receiving can be contacted directly at (240) 344-5697 (Rachel Andrews) or (301) 401-8070 (Amy Pantella).

8.4 Use the PD Sample Shipping Manifest template in Appendix 2 to generate a shipping list containing pertinent sample information.

8.5 Make a copy of the Shipping Manifest and specimen Batch Records so that one copy can be sent to FNLCR with the biopsy samples and one copy can be maintained at the collection site for internal records.

8.6 Day of Shipment

8.6.1 Just prior to shipment, place specimen tubes into a biohazard specimen bag then in an insulating Styrofoam shipping container. The insulating Styrofoam shipping containers are required to have dimensions of ***at least*** 14″×11″×9″ (length, width, height) with a ***minimum*** of 20 pounds of added dry ice. Sufficient dry ice is imperative to maintain the samples at -20°C for at least 72 hours. Expect 10 pounds of dry ice to sublimate per day during transit. Add additional dry ice to the required 20 pounds if shipping is anticipated to be longer than 24 hours.

8.6.2 All weekly processing specimens are recommended to ship out on Mondays through Thursdays via FedEx Priority Overnight (excluding any day before a federal holiday).

8.6.3 **Verify** that the contents of the package match the Shipping Manifest and sign and date the bottom of both copies of the Shipping Manifest. Place one copy of the Shipping Manifest inside the shipping box along with copies of the completed Batch Records for all specimens.

8.6.4 Seal the box and print and attach the shipping address onto the outside of the shipping container; be sure the container is labeled as containing biohazardous specimens.

8.6.5 Record the shipping date, time, tracking number, and shipping information in the Batch Record (Appendix 1, Section 4).

8.6.6 E-mail FNLCR PD Specimen Central Receiving (NCI_PD_Support@mail.nih.gov) a shipment notification. State "*Protocol Name* PD Specimen Shipment" in the subject line and reference the tracking number in the e-mail. Please notify FNLCR PD Specimen Central Receiving of any issues or protocol deviations as soon as possible and provide written notes on the Batch Record.

8.6.7 Once specimens arrive at the receiving laboratory, they should be immediately placed at -80ºC (or lower) pending delivery to the processing laboratory for protein extraction.

APPENDIX 1: BATCH RECORD

Place PD SpecimenLabel Here

A **separate** Batch Record should be started for **each patient sample set**.

Note*:* A pre-dose and post-dose sample from the same patient would have the same Patient ID, but different Specimen ID numbers.

Note: Record times using **military time** (24-h designation); for example, specify 16:15 to indicate 4:15 PM.

Certified Assay Operator: _____

Certification Number: _____

☐ Check here if PK/PD Support Lab Personnel

Facility/Clinic Collecting Specimens: _____

Clinical Protocol Number: _____

Patient ID: _____

Primary Diagnosis: _____

1. Biopsy Collection

	1st Pass	2nd Pass	3rd Pass	4th Pass
Specimen ID				
Site of Biopsy (complete for all passes or note "same" for replicate cores)				
Description of Biopsy (*e.g.*, large intact core or small and fragmented core)				
Biopsy Timepoint (Cycle, Day, and Hours post dose, if post treatment)				
Biopsy size prepared for PD or histological analysis:	☐ Full ☐ Halved	☐ Full ☐ Halved	☐ Full ☐ Halved	☐ Full ☐ Halved
Required: Time elapsed from collection to placement in tube	min sec	min sec	min sec	min sec
Time biopsy collected (opt)	:	:	:	:
Time biopsy placed in tube (opt)	:	:	:	:

2. Biopsy Procedure Details

Specimen ID	
Time local anesthesia administered	:
Dose of local anesthetic	mg
Name of local anesthetic used (from Research Nurse)	
Time of skin incision	:
Needle Type (*e.g.*, Temno)	
Needle diameter	gauge
Needle Length	cm
Time guide needle introduced	:
Time guide needle placement confirmed	:
Time biopsy needle introduced	:

3. Biopsy Storage

Date/time biopsy specimen(s) placed at -80°C (or lower) _____/ /_____ _____:_____ _____ºC

4. Shipping to FNLCR (optional)

Date and time samples shipped _____ _____:_____

Tracking information _____

****Attach copy of Shipping Manifest**

5. Notes, including any deviations from the SOP:

6. Laboratory Director/Supervisor Review of Batch Record

Laboratory Director/Supervisor:_____(PRINT)

_____(SIGN)

Date: ____/____/_____

APPENDIX 2: PD SAMPLE SHIPPING MANIFEST

From:

Phone:
Email:

PD Sample Shipping Manifest

In Package	Item No	Patient/ Specimen ID	Clinical Protocol	Description	Primary Diagnosis	Site of Biopsy	Time Point Scheduled	Collection Date	Collection Time
	Example	*1234-xx999-1023-500*	*12-C-0000*	*Full biopsy*	*Melanoma*	*Right forearm*	*Pre-dose D1*	*06/12/12*	*08:50*
	Example	*1234-xx999-1023-501*	*12-C-0000*	*Half biopsy*	*Melanoma*	*Right forearm (same lesion)*	*Cycle 1, D8*	*06/20/12*	*16:05*
	1							/ /	
	2							/ /	
	3							/ /	
	4							/ /	
	5							/ /	
	6							/ /	
	7							/ /	
	8							/ /	
	9							/ /	
	10							/ /	

Verification of Contents **Signature** **Date**

Contents Verified Collection Laboratory _____ / /

Contents Verified FNLCR PD Central Receiving _____ / /