To: CTEP Protocol and Information Office

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Branch: Investigational Drug Branch (IDB), CTEP, DCTD, NCI

Date: August 26, 2024

Re: Review of Protocol #10403: "Phase 1 Trial of Gemcitabine Combined with the Elimusertib (BAY 1895344) ATR Inhibitor with Expansion Cohorts in Advanced Pancreatic and Ovarian Cancer"

SUMMARY OF CHANGES – Protocol

#	Section	Comments
1	10.3.3	- Expedited AE reporting requirements table updated.

NCI Protocol #: 10403

Local Protocol #:

ClinicalTrials.gov Identifier: NCT04616534

TITLE: Phase 1 Trial of Gemcitabine Combined with the Elimusertib (BAY 1895344) ATR Inhibitor with Expansion Cohorts in Advanced Pancreatic and Ovarian Cancer

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Participating Organizations: Dose Escalation

LAO-MA036 / Dana-Farber - Harvard Cancer Center LAO
LAO-CT018 / Yale University Cancer Center LAO
LAO-NCI / National Cancer Institute LAO

Participating Organizations: Dose Expansion

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
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NCI-Supplied Agent(s): Elimusertib (BAY 1895344) (NSC 810486)
Other Agent(s): Gemcitabine hydrochloride (NSC 613327), Commercial

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

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Revision 1 / October 5, 2020
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Amendment 2 / March 7, 2022
Amendment 3 / May 12, 2022
Amendment 4 / April 21, 2023
Amendment 5 / February 26, 2024
Amendment 6 / August 26, 2024

SCHEMA

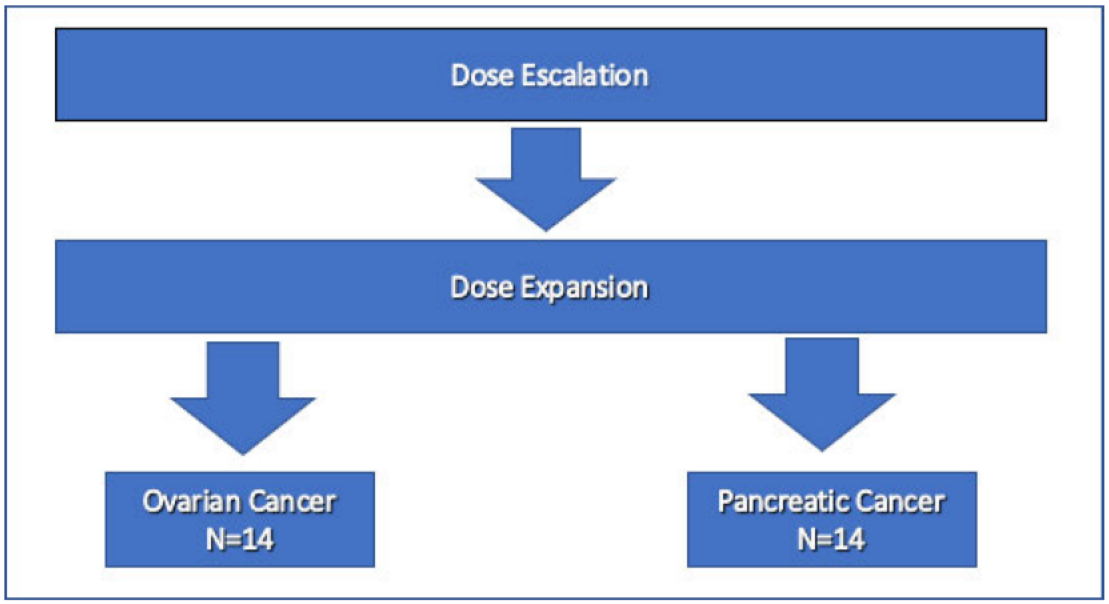

Figure 1: Gemcitabine/ Elimusertib (BAY 1895344) trial schema

Table 1:Dose Escalation

Dose Level	Gemcitabine Dose	Cycle Length
-1	100mg/m2 (Day 1 and 8)	21 days
1	175mg/m2 (Day 1 and 8)	21 days
2	250mg/m2 (Day 1 and 8)	21 days
3	350mg/m2 (Day 1 and 8)	21 days
4	500mg/m2 (Day 1 and 8)	21 days

Dose Level	Elimusertib (BAY1895344)	Cycle Length
-2	10mg once daily (Days 2-3 and Days 9-10)	21 days
-1	20mg once daily (Days 2-3 and Days 9-10)	21 days
1	20mg twice daily (Days 2-3 and Days 9-10)	21 days
2	30mg twice daily (Days 2-3 and Days 9-10)	21 days
3	40mg twice daily (Days 2-3 and Days 9-10)	21 days

Table 2: Dose Expansion

Agent	Dose	Route	Schedule	Cycle Length
Gemcitabine	RP2D	IV	D1, 8	21 days
Elimusertib (BAY 1895344)	RP2D	PO	BID, D2-3, D9-10	21 days
RP2D = Recommended Phase 2 Dose, IV = Intravenous, PO = Orally, BID = Twice daily				

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1. OBJECTIVES

1.1 Primary Objectives

1.1.1 Dose Escalation and Expansion Cohort

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1.1.2 Dose Escalation Cohort

1.1.2.1 Determine the maximum tolerated dose (MTD) of gemcitabine in combination with elimusertib (BAY 1895344)

1.2 Secondary Objectives

1.2.1 To observe and record anti-tumor activity. Although the clinical benefit of these drugs 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 Analyze the pharmacokinetic (PK) profile of the gemcitabine and elimusertib (BAY 1895344) combination.

1.2.3 Assessing whether immunohistochemical markers of DNA damage, ɣH2AX and pNBS1, increase in on-treatment biopsies compared to the levels seen in pre-treatment biopsies

1.3 Exploratory Objectives

1.2.4 Explore biomarkers that predict response to this combination

1.2.5 Evaluate mechanisms of acquired resistance to this combination

2. BACKGROUND

2.1 Study Disease(s)

2.1.1 ATR is a Master Regulator of DNA Repair and Replication

The inherent genomic instability of many malignancies makes inhibition of DNA repair proteins an attractive treatment strategy. The success of poly-ADP ribose polymerase (PARP) inhibitors in breast, ovarian and, more recently, pancreatic cancer demonstrates the promise of this approach. Although the clinical development of PARP inhibitors was a major step forward, preclinical

studies have demonstrated multiple additional targets along the DNA repair pathway. One of the most promising of these targets is a member of the PI3K-related kinase (PIKK) family called ataxia telangiectasia and RAD3 related protein (ATR).

ATR plays a critical role in double stranded DNA repair, a process also called homologous recombination (HR). In addition, ATR plays a major role in activating the regulatory processes that safeguard DNA replication. ATR is activated when endogenous or exogenous factors prevent DNA replication forks from duplicating DNA in S-phase. Stalling or collapse of DNA replication forks, termed replicative stress, is typically compensated for by activation of ATR, thus resulting in prolongation of the S/G2 phase of the cell cycle (Gelot *et al*., 2015; Dobbelstein and Sorensen, 2015). When a replication fork becomes unstable, because of factors such as DNA damage or a lack of deoxyribonucleotide triphosphates (dNTPs), single stranded DNA in the stalled replication fork becomes coated with phosphorylated-human replication protein A (pRPA) (Dobbelstein and Sorensen, 2015; Bhat & Cortez, 2018). The presence of pRPA leads to the activation of the regulatory kinases ATR and its downstream effector CHK1, which govern the cellular response to replicative stress.

2.2 CTEP IND Agent

2.2.1 Elimusertib (BAY 1895344)

DNA-damage response (DDR) is a multicomplex network of signaling pathways involved in detection and repair of DNA damage, transient cell cycle arrest to ensure genomic stability and cell viability (Fokas *et al.*, 2014; Zeman and Cimprich, 2014; Weber and Ryan, 2015). Deficiencies in DDR mechanisms have been shown to contribute to tumor development. Ataxia telangiectasia mutated (ATM) and ataxia telangiectasia and Rad3-related (ATR) protein kinases are key regulators of DDR. They both contribute to maintaining genome integrity in response to various exogenous and endogenous genotoxic insults, *e.g.*, cytotoxic chemotherapy, ultraviolet light, ionizing radiation, or hypoxia (Fokas *et al.*, 2014; Pitts *et al.*, 2014; Yan *et al.*, 2014). Although ATR and ATM have broadly overlapping substrate specificities, they have non-redundant functions, which are well coordinated during DDR. ATR is a key replication-stress-response kinase (Cimprich and Cortez, 2008; Zeman and Cimprich, 2014; Fokas *et al.*, 2014; Wengner *et al*., 2020). While ATM is primarily activated by DNA double-strand breaks (DSBs), ATR is recruited to regions of replication protein A (RPA)-coated ssDNA generated at stalled replication forks or during the of processing DSBs (Menezes *et al.,* 2014; Reaper *et al*., 2011; Yan *et al.,* 2014). ATR phosphorylates/activates checkpoint kinase 1 (CHK1) at serine 345 ($CHK1pS^{345}$), which stabilizes stalled replication forks until replication stress is resolved and DNA damage is repaired (Fokas *et al.*, 2014; Pitts *et al.*, 2014). Unlike normal cells, cancer cells are often deficient in ATM signaling, which renders them more reliant on the ATR-controlled S/G2-phase checkpoints for repairing DNA damage and survival (Fokas *et al*., 2014, Menezes *et al*., 2015; Reaper *et al*., 2011). ATR inhibition in the ATM-signaling deficient tumors results in synthetic lethality. Preclinical data suggest therapeutic utility of ATR inhibitors for cancer treatment as they may mediate synthetic lethality in DDR-deficient tumors and in combination with DNA-damaging agents (*e.g*., radiation, cytotoxic chemotherapy), targeted therapies compromising DNA-damage repair, such as poly(ADP-ribose)polymerase (PARP) inhibitors or anti-androgen therapies may enhance their activity (Wengner *et al.,* 2020)

2.2.1.1 Nonclinical Studies

Nonclinical *In Vitro* Activity and Mechanism of Action

Elimusertib (BAY 1895344) is a highly potent and selective inhibitor of ATR kinase activity, with a 50% inhibitory concentration (IC_{50}) of 7 nmol/L of this enzyme (Investigator's Brochure, 2020; Luecking *et al.,* 2017). The activity and selectivity of elimusertib (BAY 1895344) were evaluated in biochemical assays against a panel of 395 kinases (Investigator's Brochure, 2020). Mechanistic target of rapamycin (mTOR) was identified as the most sensitive off-target kinase (IC_{50}=35 nmol/L). Elimusertib (BAY 1895344) inhibited proliferation of a broad spectrum of human tumor cell lines with a median IC_{50}=78 nmol/L (mean IC_{50}=150 nmol/L). A clear separation between highly sensitive (IC_{50} <100 nmol/L) and less sensitive cell lines was observed. The majority of sensitive cell lines were characterized by mutations affecting the ATM pathway. Lymphoma cell lines appeared to be most sensitive with IC_{50s} ranging from 8.6 to 32 nmol/L. Elimusertib (BAY 1895344) effects against lymphoma cell lines were mostly cytotoxic, with induction of apoptosis observed in 61% of the tested cell lines (Gaudio *et al*., 2019).

Elimusertib (BAY 1895344) activity was evaluated in cellular mechanistic assays specific for inhibition of kinase activity of ATR, ATM, or DNA-dependent protein kinase (DNA-PK) catalytic subunits (DNA-PKcs) (Investigator's Brochure, 2020). The cellular level of phosphorylated H2AX (γH2AX) was used to monitor kinase activity of ATR, ATM and DNA-PKcs. By selection of appropriate cells and stimuli to induce either DSBs or replication stress, γH2AX served as a specific read-out for the activity of ATR, ATM or DNA-PKcs. In the ATR-specific mechanistic assay in HT-29 cells Elimusertib (BAY 1895344) inhibited hydroxyurea-induced γH2AX with an IC_{50} of 36 nmol/L. Elimusertib (BAY 1895344) did not inhibit ATM- or DNA-PKcs-mediated H2AX phosphorylation up to a concentration of 10 mcmol/L.

Nonclinical *In Vivo* Activity

In vivo, elimusertib (BAY 1895344) was evaluated in several human tumor xenograft models of different tumor indications (prostate cancer, mantle cell lymphoma [MCL], diffuse large B-cell lymphoma [DLBCL], cervical cancer, lung cancer, ovarian cancer, melanoma, colorectal cancer [CRC]) in mice (Investigator's Brochure, 2020). Elimusertib (BAY 1895344) demonstrated strong antitumor activity with good tolerability as monotherapy at a dose of 50 mg/kg administered orally (PO) twice a day (BID) for 3 days followed by 4 days off treatment in biomarker-positive tumor models.

Elimusertib (BAY 1895344) had superior antitumor effects compared to other ATR inhibitors (AZD-6738, VX-970, or VX-803) as well as a PARP inhibitor, olaparib, in several tumor models. Elimusertib (BAY 1895344) showed synergistic antitumor effects in combination with DNA damage inducing therapy, such as chemotherapy (cisplatin, carboplatin, oxaliplatin, 5-FU, irinotecan) (Investigator's Brochure, 2020), α-radiation by radium-223 dichloride (Wengner *et al*., 2018; Bannik *et al.*, 2019) or thorium 227 (Wickstroem *et al.*, 2019a; Wickstroem *et al.*, 2019b), external beam radiation therapy [EBRT] (Wengner *et al*., 2020), therapies compromising DNA damage repair such as a PARP inhibitor olaparib (Wengner *et al*., 2020) or anti-androgen agent darolutamide (Wengner *et al*., 2020), therapies targeting a programmed cell death protein 1 (PD1], PD ligand 1 (PD-L1), or other targeted therapies (PI3K inhibitor) (Investigator's Brochure, 2020).

Nonclinical Pharmacology
Following single intravenous administration of elimusertib (BAY 1895344), a high volume of distribution (V_d) was observed in all species (mice, rats, and dogs) (Investigator's Brochure, 2020). In mice and dogs, elimination of the drug was bi-phasic with a short initial (distribution phase) half-life ($t_{1/2}$) of 0.2 and 1 hour, respectively, followed by a long elimination $t_{1/2}$ of 12.2 and 8.3 hours, respectively. In rats only the distribution phase $t_{1/2}$ (1.3 hours) was observed.

Following single oral dosing ofelimusertib (BAY 1895344), the elimination $t_{1/2}$ was intermediate to long in rats and could not be calculated in dogs (Investigator's Brochure, 2020). In single dosing studies in dogs, plasma exposure measured as an area under s concentration time curve (AUC) increased more than dose-proportionally over the dose range of 3-8 mg/kg, whereas a maximum concentration (C_{max}) increased dose-proportionally. After multiple oral dosing of elimusertib (BAY 1895344), AUC and C_{max} on day 17 were lower than on day 1 in rats. In dogs, exposure increased only slightly between days 1 and 23. Oral bioavailability was moderate to high (67%- 87%) in rats and moderate in dogs (51%). The binding of elimusertib (BAY 1895344) to plasma proteins was low to moderate in all investigated species. A slight concentration dependency was observed with a decrease in protein binding at the highest test concentrations (increase in the unbound fraction from 2.6% to 4.2% in humans).

Hepatic route appears to be the key clearance mechanism for this compound (Investigator's Brochure, 2020). Metabolic pathways were highly comparable across all investigated species. In human hepatocytes, elimusertib (BAY 1895344) was metabolized *via* oxidation and dealkylation at the methylmorpholine moiety and direct glucuronidation. Oxidative metabolism was mainlycatalyzed by cytochrome P450 (CYP) isoform 3A4 (CYP3A4). Elimusertib (BAY 1895344) appears to have a potential to inhibit as well as induce CYP3A4. Elimusertib (BAY 1895344) showed the inhibitory potential towards protein transporters, such as P-glycoprotein (P-gp) and breast cancer resistance protein (BCRP), with IC_{50} of 7.4 mcM and 1.9 mcM, respectively. Elimusertib (BAY 1895344) had the inhibitory potential toward organic anion transporting polypeptide (OATP)1B1 and OAT1B3.

Nonclinical Safety and Toxicology
Safety pharmacology studies revealed no effect on the human ether-a-go-go-related (hERG) potassium tail current was observed (Investigator's Brochure, 2020). No clinically relevant or major effects were observed on the central nervous system (CNS). Cardiovascular examinations revealed a moderate increase in diastolic blood pressure without clear effects on systolic blood pressure. ECG intervals were not substantially influenced.

Major target organs for elimusertib (BAY 1895344) in oral repeat-dose toxicity studies in rats and dogs with continuous and intermittent (3 days on/4 days off) dosing were the lymphatic organs and bone marrow, epithelial tissues including skin and gastrointestinal (GI) tract, and male reproductive organs (Investigator's Brochure, 2020). Additional toxicity observed in adult animals was atrophy of bone spongiosa in both species. All findings were reversible.

Elimusertib (BAY 1895344) showed genotoxicity *in vitro* (micronucleus test in Chinese hamster cells for aneugenicity and clastogenicity) and *in vivo* (bone marrow [BM] micronucleus test in rats after a 4-week repeat dosing) (Investigator's Brochure, 2020).

No specific studies on reproductive and developmental toxicity of elimusertib (BAY 1895344) were conducted at this stage of development. (Investigator's Brochure, 2020). Degenerative findings in male reproductive organs were seen in 4-week repeat-dose toxicity studies in rats and dogs. Thus, an influence on fertility can be expected. Homozygous ATR know-out mice are embryo- lethal, indicating that ATR is essential in embryogenesis. Based on its mode of action, elimusertib (BAY 1895344) is regarded as a potential teratogen.

There was an indication for phototoxicity in an *in vitro* assay (Investigator's Brochure, 2020). Overall, elimusertib (BAY 1895344) displayed an acceptable nonclinical safety profile to initiate clinical development in patients with advanced disease (Investigator's Brochure, 2020). The toxicities observed are regarded as monitorable and/or manageable in humans.

2.2.1.2 Clinical studies

Preliminary clinical experience is derived from the ongoing company-sponsored First-in-Human (FiH) study of elimusertib (BAY 1895344) (data cut-off date June 16, 2020) (Investigator's Brochure, 2020).

Clinical Pharmacokinetics
Preliminary PK show that elimusertib (BAY 1895344) administered at the maximum tolerated dose (MTD) of 40 mg PO BID for 3 days every week (3 days on/4 days off) in patients with advanced solid tumors was absorbed rapidly with a median time to reach maximum drug concentration (t_{max}) of 1 hour and a geometric mean terminal $t_{1/2}$ of 9.8 hours in plasma (Investigator's Brochure, 2020). Exposure increase was observed to be broadly dose-proportional across the dose range investigated (5-80 mg), and accumulation was consistent with observed $t_{1/2}$. Clinical exposure was observed to be in the range associated with efficacy in nonclinical models.

Clinical Safety
A. Dose-escalation cohort
Dose-limiting toxicities were observed with elimusertib (BAY 1895344) administered at doses 60 mg BID and 80 mg BID, as well as on the intermittent schedule of 60 mg BID 3 days on/4 days weekly for 2 weeks of a 3-week cycle (Investigator's Brochure, 2020). Elimusertib (BAY 1895344) administered at 40 mg PO BID on the 3-day-on/4-day-off schedule every week on a 3-week cycle has been declared the MTD.

All 39 patients who started elimusertib (BAY 1895344) treatment in the dose-escalation phase (as of June 16, 2019) experienced at least one treatment-emergent adverse event (TEAE) and 94.9% of patients experienced at least one elimusertib (BAY 1895344)-related TEAE of any grade; 61.5% had grade 3, and 17.9% had grade 4 (Investigator's Brochure, 2020). The most common study drug-related TEAEs by (occurring in ≥10% of patients) were: anemia (74.4%), neutropenia (46.2%), fatigue, nausea (38.5% each), white blood cell (WBC) count decreased (28.2%), platelet count decreased (23.1%), thrombocytopenia, and neutrophil count decreased (20.5%), diarrhea, decreased appetite (15.4% each), and vomiting (12.8%). Overall, 35.9% of patients experienced at least one treatment-emergent serious adverse event (TESAE) in the dose-escalation phase and 7.7% of patients experienced at least one elimusertib (BAY 1895344)-related TESAE: grade 4 neutropenia, grade 3 diarrhea, nausea, hypotension, and grade 2 pyrexia

2.6% each). One patient (80 mg BID cohort) died within 30 days of treatment discontinuation, but the death was assessed as unrelated to the study drug.

B. Expansion cohort
At least one TEAE was observed in 97.4% of patients (75/77) treated at the MTD and at least one elimusertib (BAY 1895344)-related TEAE of any grade was experienced by 88.3% of patients, grade 3 (in 62.3% of patients), and grade 4 (in 13.0% of patients) (Investigator's Brochure, 2019). The most common drug related TEAEs (occurring in ≥10% patients) were: anemia (71.4%), neutropenia (41.6%); fatigue (28.6%), thrombocytopenia, nausea (18.2% each), WBC count decreased (15.6%), neutrophil count decreased (11.7%), and vomiting (10.4%). Four patients (5.2%) experienced at least one elimusertib (BAY 1895344)-related TESAE. There were three deaths in the expansion cohort, occurring within 30 days of permanent treatment discontinuation and all three were considered unrelated to the study drug.

2.3 Other Agent

2.3.1 Gemcitabine

Gemcitabine is a masked-chain-terminating nucleoside analogue that kills cells undergoing DNA synthesis and blocks the progression of cells through the G1/S-phase boundary. It is metabolized to diphosphate and triphosphate forms. Gemcitabine diphosphate inhibits the enzymatic generation of deoxynucleoside triphosphates (dNTPs) for DNA synthesis, resulting in reductions in dNTP concentrations, including dCTP. Gemcitabine triphosphate competes with dCTP for incorporation into DNA. Together, these activities enhance the incorporation of gemcitabine triphosphate into DNA, blocking further DNA synthesis and resulting in apoptosis (Gemcitabine Package Insert, 2019). Gemcitabine as a single agent or combined with other chemotherapies is FDA-approved for the treatment of relapsed ovarian cancer, metastatic breast cancer, non-small cell lung cancer, and pancreatic cancer.

2.4 Rationale

2.4.1 Elimusertib (BAY1895344) ATR Inhibitor

Several ATR inhibitors (AZD6738, VX-970 andelimusertib (BAY1895344)) are currently in clinical development. Elimusertib (BAY1895344) is a selective and potent inhibitor of ATR with an IC_{50} of 7 nM. Unlike AZD6738 and VX-970, elimusertib (BAY1895344) has shown activity in an ATM deficient murine model of mantle cell lymphoma. The results of the phase 1 trial of elimusertib (BAY1895344) have demonstrated that this compound is reasonably well tolerated and has encouraging clinical activity. The dose limiting toxicity is myelosuppression, and the maximum tolerated dose (MTD) of elimusertib (BAY1895344) is 40 mg twice daily, with 3 days on/4 days off continuously. Three patients with ATM mutated tumors achieved a partial response on elimusertib (BAY1895344). A partial response was also observed in one patient with BRCA1 mutated ovarian cancer, whereas another patient with a heavily pretreated BRCA1 mutated ovarian cancer, who had previously been treated with a PARPinhibitor, had a tumor reduction of 20% and continued the trial for more than 1 year (De Bono*et al*, 2019).

The ongoing development of several ATR inhibitors has led investigators to identify several

genomic biomarkers of ATR inhibitor sensitivity. Multiple groups have demonstrated that amplification of cyclin E and MYC, proteins well known to increase DNA replication, increases susceptibility to ATR inhibition (Jones *et al*., 2017; Toledo *et al*., 2011; Murga *et al*., 2011; Buisson *et al*., 2015). Preclinical studies have also identified deleterious ARID1A and POLD1 mutations as potential biomarkers for sensitivity to ATR inhibition (Hocke *et al*., 2016; Williamson *et al*., 2016). Other potential biomarkers of ATR inhibitor sensitivity include DNA repair genes. Several investigators have observed that combined deficiency of ATM and ATR function leads to synthetic lethality (Kwok *et al*., 2016; Min *et al.,* 2017). Emerging data indicate that mutations in other DNA repair genes also increase ATR inhibitor sensitivity. In agreement with this finding, two BRCA1 mutated patients showed a significant clinical benefit in the elimusertib (BAY1895344) phase 1 trial. An analysis of PARP resistant ovarian cancers showed that ATR inhibition can re-sensitize BRCA1 mutated tumors to PARP inhibition (Yazinski *et al*., 2017).

2.4.2 Rationale for Therapies Targeting Replicative Stress

Therapies that inhibit cancer cells' ability to compensate for replicative stress, are particularly attractive in treating cancers with replicative stress generating genomic alterations, because they interfere with DNA repair and rapid cell growth (Dobbelstein and Sørensen, 2015). For example, a sizable number of pancreatic cancers have DNA repair deficiency and harbor *KRAS* mutations, *CDKN2AB* loss and TP53 mutations (Kotsantis *et al*., 2018). Moreover, 5% and 3% of pancreatic cancers harbor *MYC* and *CCNE1* amplifications, respectively, which are also known to generate high levels of replicative stress (Aguirre *et al*., 2018).

Although some cancers have an inherent degree of endogenous replicative stress, an active area of research is exploring ways to exogenously increase replicative stress. One widely recognized means of increasing replicative stress is creating conditions that decrease the cellular concentration of dNTPs, by using drugs such as hydroxyurea and gemcitabine (Ubhi and Brown, 2019). Depriving growing replication forks of dNTPs deprives DNA of its necessary building blocks and results in substantial replicative stress. Gemcitabine, a nucleoside analog commonly used in treating patients with pancreatic and ovarian cancer, exerts most of its cytotoxicity through incorporation of its metabolite dFdCTP into DNA, thus resulting in replication strand termination (Brunton *et al*., 2011). However, gemcitabine also targets cancer cells by irreversibly inhibiting ribonucleotide reductase (Brunton *et al*., 2011). This inhibition of ribonucleotide reductase, an enzyme that produces dNTPs, results in decreased dNTP concentrations and creates replicative stress (Ubhi and Brown, 2019). Of note, in a cellular adaptation to this gemcitabine-induced irreversible inhibition of ribonucleotide reductase, gemcitabine treatment has been found to result in compensatory upregulation of the expression of RRM2, the catalytic subunit of ribonucleotide reductase (Liu *et al*., 2017).

Leveraging the ability of gemcitabine to generate replicative stress, several ongoing clinical trials of CHK1 inhibitors have used the strategy of combining gemcitabine with CHK1 inhibition. This strategy is based on preclinical work demonstrating that gemcitabine has strong synergy with CHK1 inhibitors (Drápela *et al*, 2020). For example, in the pancreatic ductal

adenocarcinoma (PDAC) cell line MiaPaCa2, Bliss analysis has demonstrated that gemcitabine potentiates the activity of the CHK1 inhibitor GNE-783 by 185-fold; similarly, the related CHK1 inhibitor GNE-900 shifts the half-maximal growth inhibitory effect (GI_{50}) of gemcitabine by 93-fold (Xiao *et al*., 2013). This strong synergy suggests that CHK1 inhibitors may be combined with low-dose gemcitabine. In this strategy, low-dose gemcitabine generates replicative stress and primes cancer cells to respond to the CHK1 inhibitor. An additional advantage of this strategy is that low doses of gemcitabine cause less myelosuppression. At ASCO 2019, data from a phase 1 trial combining the SRA737 CHK1 inhibitor with low-dose gemcitabine were presented. In this trial, low doses of gemcitabine at 250 mg/m^2 on days 1, 8, and 15 of a 28-day cycle were able to be safely administered with SRA737 (Banerji *et al*., 2019). Despite this low dose of gemcitabine, confirmed partial responses were observed in anogenital cancers and ovarian cancer (Banerji *et al*., 2019). Notably, partial responses were not observed in a separate trial testing single-agent SRA737 (Plummer *et al*., 2019).

Similarly, to that of CHK1, the critical role of ATR in counteracting replicative stress suggests that combining ATR inhibition with gemcitabine might also be an effective strategy (Figure 2). In addition to stabilizing replication forks, in response to replicative stress, ATR increases RRM2 levels, thereby ensuring adequate levels of dNTPs (Buisson *et al*., 2015). Hence, ATR inhibition would prevent compensatory increases in RRM2 after its suppression by gemcitabine. Notably ribonucleotide reductase overexpression is a mechanism of resistance to gemcitabine, and overexpression of ribonucleotide reductase doubles the lifespan of ATR-deficient mice (Lopez-Contreras *et al*., 2015; Qin *et al*., 2016; Voutsadakis, 2011).

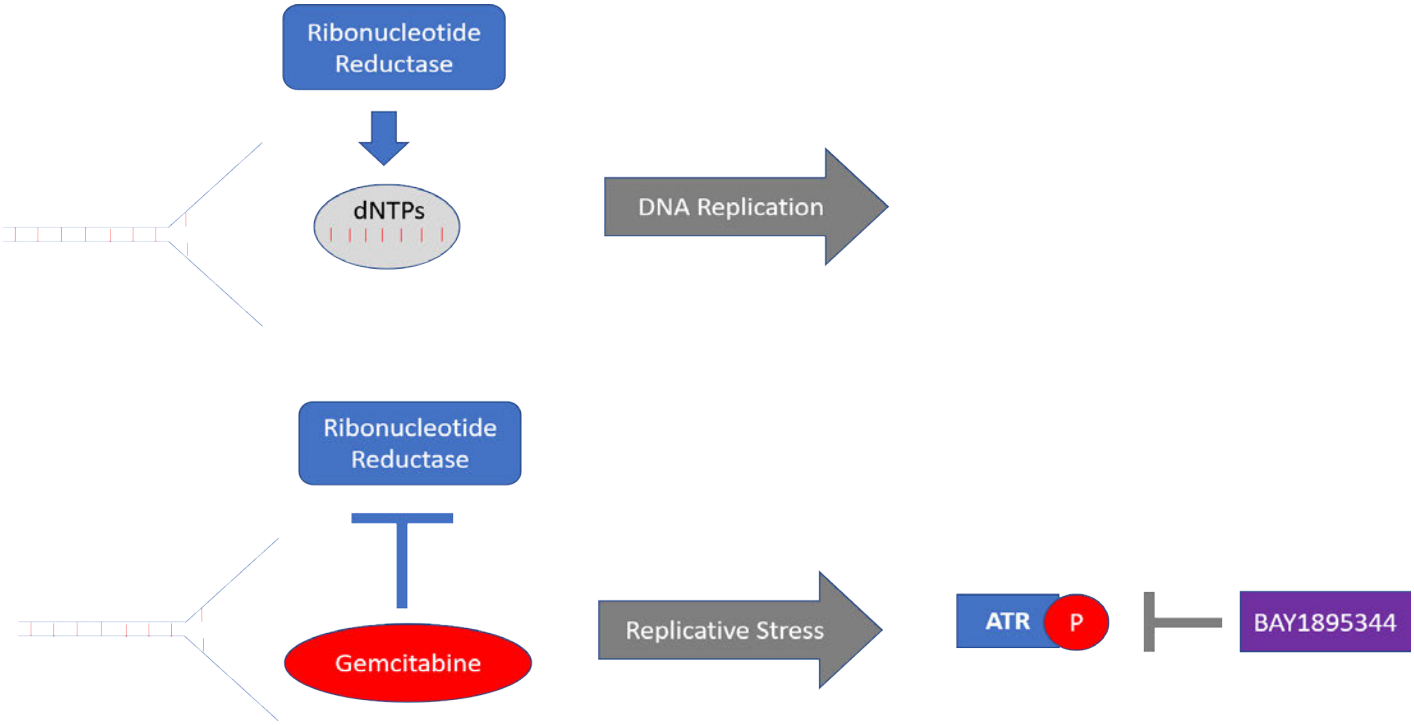


Figure 2: Schematic of clinical trials designed to exacerbate replication stress and preventcompensatory mechanisms for increased replication stress in PDAC. *KRAS mutation,*

***CDKN2A/B* loss and *TP53* loss produce endogenous replication stress in PDAC cells. This stresscan be exacerbated by gemcitabine, a potent inhibitor of ribonucleotide reductase, thus resulting in replication fork stalling, even at low doses. The ATR-CHK1 axis is critical for stabilizing stalled replication forks, and the WEE1 kinase prevents premature mitotic entry until DNA replication is complete. Inhibition of ATR-CHK1 or WEE1 further exacerbates replication stressand induces lethality when combined with gemcitabine.**

2.4.3 Preclinical Data Supporting the Combination of Gemcitabine and ATR Inhibition

The therapeutic effectiveness of combining gemcitabine with ATR inhibition has been shown in preclinical models of multiple tumor types (Hall *et al.,* 2014; Fordham *et al*., 2018). Fordham *et al*. have demonstrated that gemcitabine acts synergistically with ATR inhibition in *in vitro* and *in vivo* models of acute myeloid leukemia (AML). Remarkably, combination treatment with gemcitabine and the VX-970 ATR inhibitor has been found to result in durable eradication of disease in an orthotopic murine model of AML (Fordham *et al*., 2018). Liu *et al*. found that, in a panel of pancreatic cancer cell lines, combining the AZ20 ATR inhibitor with gemcitabine substantially increases the amounts of DNA double-strand breaks, and inhibition of gemcitabine induces upregulation of RRM2. Furthermore, the synergistic interaction between these two drugs results in a significant decrease in the concentration of gemcitabine needed to cause cytotoxicity (Liu *et al*., 2017). In agreement with these data, we have found that the elimusertib (BAY1895344) ATR inhibitor combined with gemcitabine results in synergistic cell death in the HR proficient cell line PANC1. In PANC1 cells, we observed synergistic cytotoxicity with theelimusertib (BAY1895344)/gemcitabine combination (Figure 3).

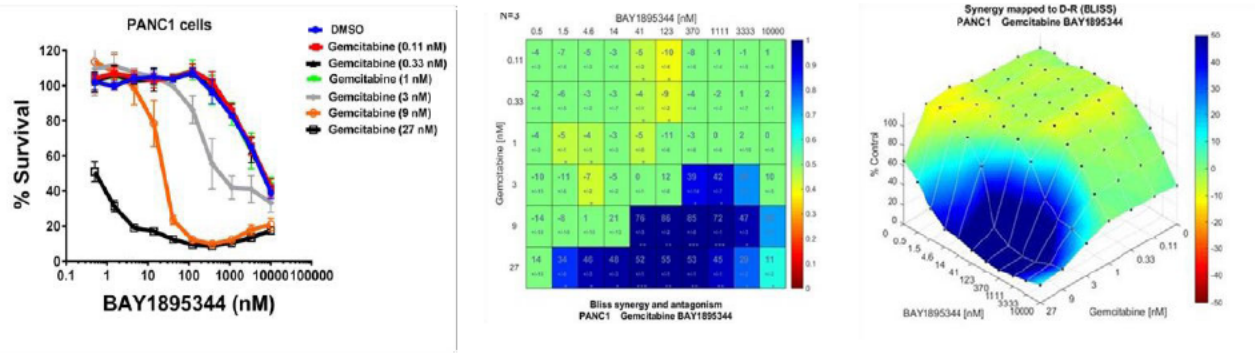


Figure 3. PANC1 cells were treated with combined gemcitabine andelimusertib (BAY1895344), demonstrating profound synergy at concentrations of gemcitabine < 10 nM. These experiments support the evaluation of low-dose gemcitabine in combination with ATR inhibition in HR-proficient pancreatic cancers.

In a comprehensive study, Wallez *et al*. have examined the mechanisms of the synergy between gemcitabine and ATR inhibition. They observed, in a panel of pancreatic cancer cells, that the AZD6738 ATR inhibitor prevents gemcitabine-induced CHK1 phosphorylation, intra-S-phase checkpoint activation and compensatory RRM2 reaccumulation. This is achieved by blocking the cancer cell's ability to compensate for the gemcitabine-induced replicative stress; ATR

inhibitor treatment markedly increases biomarkers of replicative stress (pRPA) and DNA damage (γH2AX). Similar to Liu *et al*., Wallez *et al*. observed that the concentrations of gemcitabine necessary to achieve total growth inhibition are substantially lower when the gemcitabine/ATR inhibitor combination is used, as compared with gemcitabine monotherapy.

To extend their *in vitro* findings, Wallez *et al*. tested the efficacy of the gemcitabine and AZD6738 combination in the K8484 pancreatic cancer cell line allograft model. To do so, they first performed experiments to optimize the schedule of gemcitabine and AZD6738 administration. They observed that the optimal schedule was gemcitabine administration on day 1 combined with administration of an ATR inhibitor from day 1 (after gemcitabine administration) for 136 hours. Using this schedule, they found that, in contrast to single agent AZD6738 or gemcitabine, the combination of gemcitabine and AZD6738 resulted in significant tumor shrinkage. In addition, the AZD6738/gemcitabine combination, compared with monotherapy treatment with either of the drugs, significantly improved survival of the mice. Following these experiments in the K8482 allograft murine model, they tested the gemcitabine/AZD6738 combination in an autochthonous KRAS G12D; p53 R172H; Pdx-cre (KPC) mouse model of pancreatic cancer that is notoriously difficult to treat. Impressively, they found that the combination of gemcitabine and AZD6738 resulted in better tumor control than monotherapy treatment and observed tumor shrinkage in 30% of the mice. Importantly, the investigators noted that the combination of gemcitabine/AZD6738 appeared to be well tolerated, and the mice treated with the combination therapy showed no signs of weight loss.

2.4.4 Phase 2 Clinical Trial of Gemcitabine and the M6620 (VX-970) ATR inhibitor in Gemcitabine

Recently, Konstantinopoulos *et al*. conducted a randomized phase 2 clinical trial of the M6620 (VX-970) ATR inhibitor combined with gemcitabine, compared with single agent gemcitabine in ovarian cancer. In this trial, patients with platinum refractory ovarian cancer who had received up to one therapy in the refractory setting were eligible. Patients were randomized to gemcitabine monotherapy (1000 mg/m^2 on day 1 and 8 of each 21-day cycle) or combination therapy with gemcitabine (1000 mg/m^2 on day 1 and 8 of each 21-day cycle) and M6620 (210 mg/m^2 IV on days 2 and 9 of a 21-day cycle). Combination therapy was well tolerated. The primary endpoint was progression-free survival (PFS), which was 14.7 weeks for gemcitabine monotherapy and 22.9 weeks for the combination (hazard ratio 0.57; 90% CI 0.33–0.97; one-sided log-rank p = 0.047), thus indicating that the primary endpoint was met. A pre-specified analysis in patients with a prior platinum-free interval of < 3 months *vs*. those with a platinum-free interval of 3–6 months demonstrated that the patients who derived benefit from the combination were in the group with the shorter platinum-free interval (Figure 4).

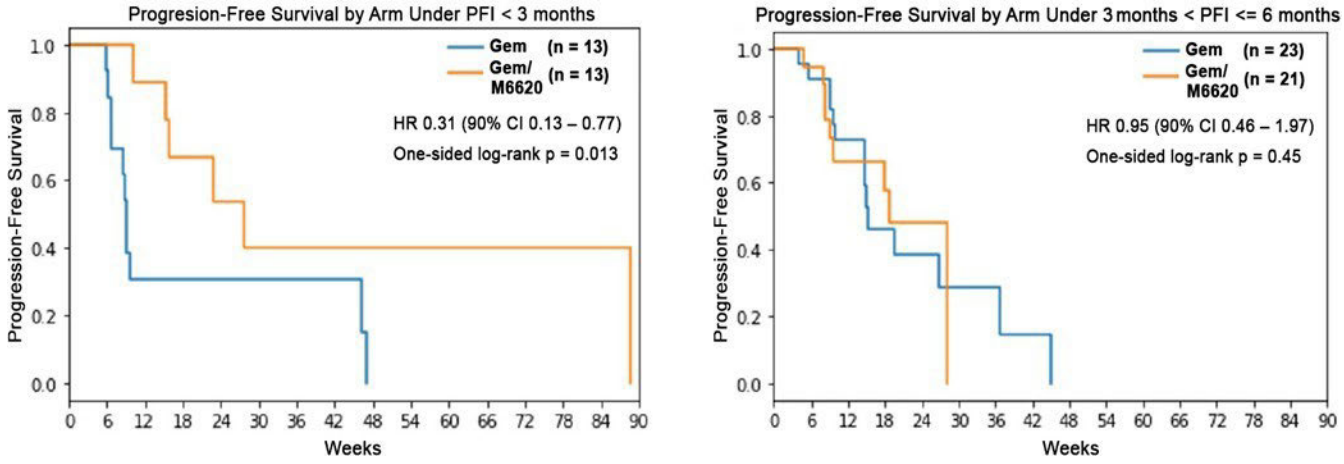


Figure 4: PFS by arm in the gemcitabine vs. gemcitabine/M6620 clinical trial. This specified stratification demonstrated that the benefit of the combination was limited to patients who had a prior platinum-free interval < 3 months. Notably, overall toxicities and response rates were equivalent between the two arms.

2.5 Rationale for Amendment Modifying the Study Schedule and Treatment Doses

In cohort 1, three patients were treated with Gemcitabine 250mg/m2 (Day 1 and 8) and elimusertib (BAY1895344) ATR inhibitor (40mg bid Days 1-3 and 8-10) of each 21-day cycle. This dose level was poorly tolerated and most toxicity was characterized by dehydration, malaise, nausea/vomiting, and fevers. Two of three patients (patient #1 and patient #2) suffered a DLT. Patient #1 had grade 4 neutropenia, grade 3 elevation of AST/ALT and grade 2 nausea and malaise. Patient #2 had grade 3 acute kidney injury, grade 3 diarrhea, grade 3 nausea, and a grade 3 pulmonary embolus. Patient #3 had grade 2 nausea/vomiting, grade 2 malaise, grade 2 elevation of AST, and grade 2 fever. While patient #1 and #2 came off the trial for toxicity (both patients' scan showed stable disease), an ovarian cancer patient (patient #3) achieved a partial response (49.7% reduction in tumor burden by RECIST criteria) and remains on the trial after more than 4 months of treatment. She's currently being treated with gemcitabine 100mg/m2 and elimusertib (BAY1895344) ATR inhibitor (20mg bid Days 1-3 and 8-12) of each 21-day cycle and is currently tolerating the study drug combination well.

Given the unexpectedly high level of toxicity, the trial is being modified to adjust the doses of the study drug. Initially the trial was designed to give the elimusertib (BAY1895344) ATR inhibitor (40mg bid Days 1-3 and 8-12) of each 21-day cycle. The schedule of the elimusertib (BAY1895344) ATR inhibitor is being modified such that it will be administered for two days (instead of three days) after each gemcitabine infusion (D2-3 and D9-10). Furthermore, since preclinical and clinical data indicates that administration of ATR inhibitor a day after gemcitabine is at least as efficacious and better tolerated, elimusertib (BAY1895344) ATR will be given the day after gemcitabine infusion (Middleton et al., 2021; Pollard et al., 2016). To be safe, dose level 1 will start with reduced dose levels of both gemcitabine (175mg/m2 on day 1 and 8) and elimusertib (BAY1895344) (20mg twice daily (Days 2-3 and Days 9-10) of each 21 day cycle.

2.6 Correlative Studies Background

2.6.1 Integrated Correlative Studies

2.6.1.1 γH2AX, pNBSI IFA with βCATN

We hypothesize that the gemcitabine/ATR inhibitor combination will increase replicative stress and cause increased DNA damage. To evaluate this, we will compare γH2AX and pNBS1

staining levels seen before treatment and then on-treatment. Our hypothesis is that the gemcitabine and ATR inhibitor combination will increase levels of both ɣH2AX and pNBS1 in the tumor.

2.6.1.2 Elimusertib (BAY 1895344) and Gemcitabine PK

Elimusertib (BAY 1895344) is a novel agent, and the proposed PK studies will further define the properties in a homogeneous population. In addition, monotherapy Elimusertib (BAY 1895344) has its own bone marrow suppressive toxicity, allowing us to explore correlations of exposure with toxicity and/or response.

We hypothesize that elimusertib (BAY 1895344) exposure correlates with toxicity.

Gemcitabine PK, though more established, has been proposed for therapeutic drug monitoring (Kozo *et al*. 2017), and after defining the PK in this trial, we will explore correlations with toxicity and response.

We hypothesize that gemcitabine exposure correlates with toxicity.

2.6.2 Exploratory Correlative Studies

2.6.2.1 Whole Exome Sequencing (Tumor Tissue)

We will perform whole exome sequencing (WES) on the pre-treatment biopsy to document the tumor's genomic makeup prior to initiating therapy. We hypothesize that tumors with genomic alterations which increase replicative stress, such as ATM and HR gene mutations as well as CCNE1 amplifications, will be more susceptible to the gemcitabine and ATR inhibitor combination. Knowledge of the genomic composition of the tumors prior to treatment will help us understand genomic determinants of sensitivity and resistance.

2.6.2.2 RNA Sequencing

We will perform RNA sequencing (RNASeq) on pre and on-treatment tumor biopsies. Our hypothesis is that signatures of replicative stress seen in pre-treatment biopsy will predict response to the gemcitabine/ATR inhibitor combination. Furthermore, we hypothesize that the RNAseq signature of replicative stress will increase in response to the gemcitabine/ATR inhibitor treatment.

2.6.2.3 RAD51

Novel RAD51 focus formation assay to identify functional HR deficiency:

A crucial event during DNA repair by HR is the formation of the RAD51 nucleofilament at sites of double-strand breaks. These structures appear as sub-nuclear foci, and the presence of RAD51 foci is a surrogate marker for HR proficiency. We have recently established an immunohistochemistry (IHC) assay for the detection of RAD51 foci to rapidly determine the functional status of HR in formalin fixed paraffin embedded (FFPE) samples. The assay was developed and standardized using breast and ovarian cancer cell lines, patient-derived xenograft (PDX) models, organoids, and primary tumor samples, including core needle biopsies. Basal levels

of RAD51 foci are observed in proliferating tumors (*i.e.*, those with at least 3% geminin positivity) and can distinguish HR-proficient from HR-deficient tumors, even in the absence of exogenous damage. As expected, irradiation of the sample increases both the number of foci per cell and the number of cells with foci.

Examples validating the assay are shown in (**Figure 5)**. Samples with deleterious *BRCA1/2* mutations or methylation of the *RAD51C* promoter lack cells with RAD51 foci, even after irradiation. Furthermore, when RAD51 foci are present in *BRCA1/2*-mutated samples, we find that HR is restored by either reversion mutation in *BRCA2* or by loss of genes involved in non-homologous end joining (*e.g.*, 53PB1), which facilitates BRCA1-independent end resection, an early step in HR. Together, these observations suggest that the RAD51 assay is a highly specific, reliable, and robust method for determining the functional status of the HR-mediated DNA repair pathway.

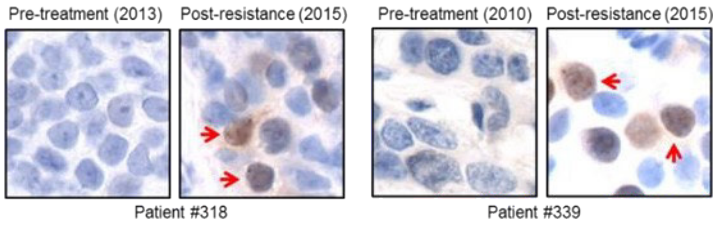


Figure 5. RAD51 assay confirms HR restoration in PARP-inhibitor-resistant metastatic breast tumors. The RAD51 assay was performed on biopsies obtained from a BRCA2 mutation carrier treated with a PARP inhibitor (#318) or a patient with tumor harboring RAD51C promoter methylation treated with carboplatin (#339). The pre-treatment samples (left) are devoid of RAD51-foci-positive cells, suggesting HR deficiency, while the therapy-resistant samples (right) are RAD51 foci-positive, suggesting HR proficiency.

2.6.2.4 pKAP1, pRPA32, pATR, SLFN11, Cyclin E, MYC

We hypothesize that immunohistochemical markers of replicative stress, pKAP1 and pRPA32, will increase as a result of treatment with the gemcitabine and ATR inhibitor combination.

We hypothesize that levels of pATR and pCHK1 will decrease as a result of treatment with the gemcitabine and aTR inhibitor combination.

In response to high levels of replicative stress, SLNF11 can signal pathways that lead to cancer cell death. We hypothesize that low levels of SLNF11 in the pre-treatment biopsy will predict resistance to the gemcitabine/ATR inhibitor combination.

Increased levels of MYC and Cyclin E can cause increased replicative stress. We hypothesize that tumors with higher levels of MYC and Cyclin E will have increased sensitivity to the gemcitabine/ATR inhibitor combination.

2.6.2.5 Organoid Cultures

Functional analysis of therapeutic sensitivity in PDAC organoid models:
We have established highly effective protocols for organoid culture of primary and metastatic PDAC biopsy specimens at DFCI and the Broad Institute. We now achieve an approximately 70% success rate in organoid culture from PDAC core biopsies. To date, we have established a

collection of over 120 patient-derived PDAC organoids that have been genetically and phenotypically characterized. These organoid cultures are typically established in culture within 6-8 weeks of biopsy. We have established robust protocols for *in vitro* sensitivity testing of FDA-approved and novel clinical trial compounds that creates the opportunity for co-clinical trials. To date, we have profiled over 75 organoids with a large panel of single agent anticancer therapies. Notably, we observed that one population of organoids is sensitive to cytotoxic chemotherapeutic agents like cisplatin and gemcitabine as well as DNA repair inhibitors like ATR and CHEK1 inhibitors, while another population is refractory to these agents (**Figure 6**).

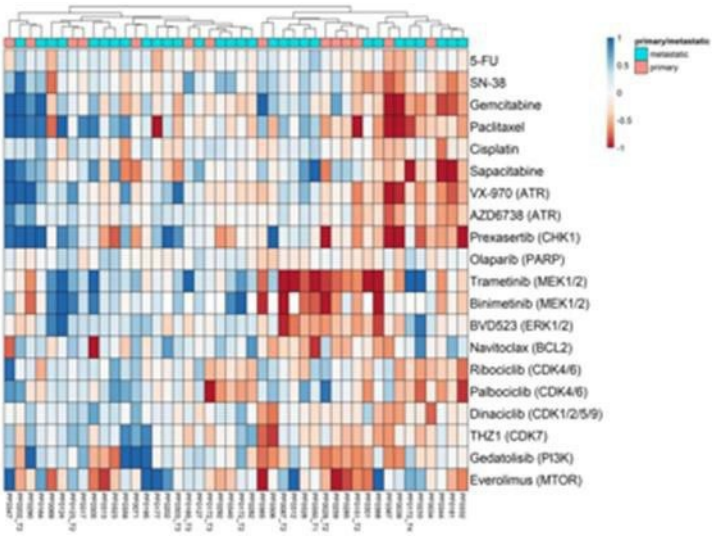


Figure 6: Testing of a PDAC organoid panel with a battery of cytotoxic agents, DNA repair inhibitor and signal transduction inhibitors. A subset of organoid cultures is highly sensitive to chemotherapy, as well as DNA repair inhibitors, including AZD6738 or VX-970-mediated ATR inhibition, or prexasertib-mediated CHK1 inhibition. Other organoids are less sensitive or insensitive, but are sensitive to other agents, including MAP kinase inhibitors. Work is ongoing to assess determinants of sensitivity to DNA damaging agents and DDR repair inhibitors.

2.6.2.6 Replication Fork Stability Assay

DNA fiber replication fork assay and IHC markers to assess replicative stress:

We have developed a DNA fiber assay to assess the stability of stalled replication forks in growing cells, and organoid cultures. In a panel of ovarian cancer organoids, we demonstrated the feasibility of using DNA fiber assays to assess DNA replication fork stability (Kais *et al*., 2016). We evaluated the ability of these organoid cells to protect stalled replication forks using DNA fiber assays (Nieminuszczy *et al*., 2016) (**Figure 7**). Along with DNA fiber assays, we and others have demonstrated IHC analyses of replication stress markers (*e.g*., p-KAP1, p-RPA2, Cyclin E, MYC), as pharmacodynamic markers for replicative stress and sensitivity to ATR inhibition (Hill *et al* 2018).

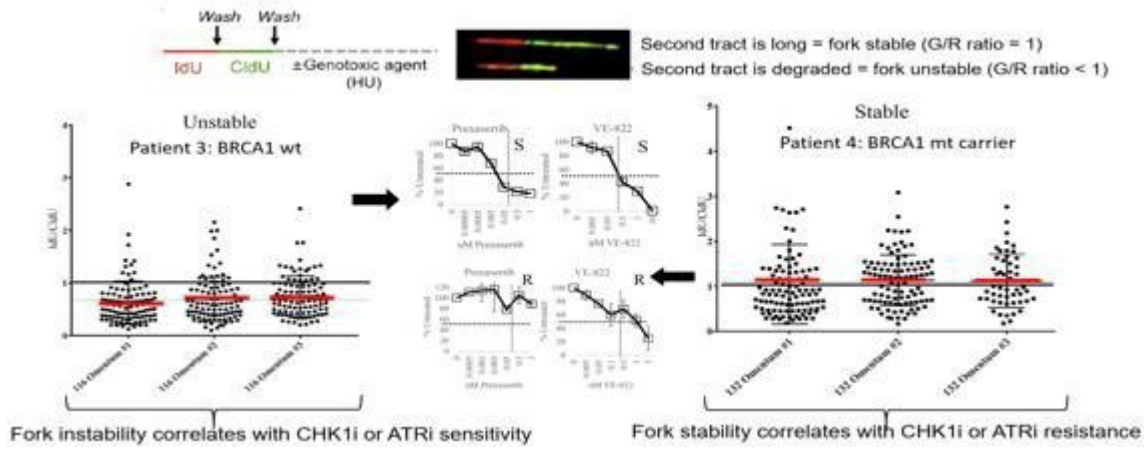


Figure 7: DNA fiber assays in ovarian organoid cultures. Organoids were sequentially pulsed for equal time periods with two nucleotide analogs, CldU and IdU, followed by exposure to fork stalling agent hydroxyurea. If the ratio of the track lengths is one, then the fork was protected during replication stalling. If the ratio of track lengths is less than one, then the fiber containing the second analog was degraded, indicating that the tumor cell was unable to protect stalled forks. In our work on ovarian cancer organoids, we demonstrated that replication fork instability correlates with sensitivity to platinum chemotherapy, ATR inhibition, and CHK1 inhibition.

2.6.2.7 ctDNA Sequencing

An important question is determining the mechanisms of resistance of the gemcitabine and ATR inhibitor combination. We will collect serial ctDNA samples so that we can analyze the mechanism of acquired resistance to this combination

2.6.2.8 ATM Expression

Preclinical evidence has demonstrated that ATM deficiency increases susceptibility to ATR inhibition. We hypothesize that tumors with ATM deficiency will be more sensitive to the gemcitabine and ATR inhibitor combination.

3. PATIENT SELECTION

3.1 Eligibility Criteria

3.1.1 Dose Escalation Cohort

3.1.1.1 Patients must have histologically confirmed solid tumor malignancy that is not curable with standard approaches. Gemcitabine must be considered a standard therapy for the participant's malignancy.

3.1.1.2 Patients must have a measurable disease, in at least one lesion, for both the dose escalation and expansion cohorts, as defined by RECIST v1.1 criteria

3.1.1.3 Patients must have received one line of treatment for their incurable cancer before

enrolling in this trial. Patients with rare malignancies for which there is no accepted standard chemotherapy regimen can enroll without any prior treatments.

3.1.1.2 Patients must not have received more than two lines of cytotoxic chemotherapy.

- Patients can have received prior gemcitabine
- Adjuvant chemotherapy is counted as one line of treatment if patients received it within 6 months of their cancer recurring.
- There is no limit for lines of prior targeted therapies or immunotherapy.
- Patients who received a prior PARP inhibitor must have had progressive disease, or intolerable toxicity, on the PARP inhibitor prior to enrolling on the study.

3.1.2 Dose Expansion Cohort

3.1.2.1 Participants must have a histologically confirmed advanced pancreatic adenocarcinoma or ovarian cancer (high grade serous ovarian, primary peritoneal or fallopian tube cancer) that is not curable with standard approaches. Patients with both metastatic pancreatic cancer and unresectable pancreatic cancer are eligible

3.1.2.2 Ovarian Cancer:

- Patients with ovarian cancer must have platinum-resistant disease, defined as progression within 6 months after the last platinum regimen.
- Patients with ovarian cancer cannot have received more than one prior regimen in the platinum-resistance setting.

3.1.2.3 Pancreatic Cancer:

- Patients with pancreatic cancer cannot have received more than one line of cytotoxic chemotherapy in the metastatic setting.
    - Adjuvant chemotherapy is not counted as one line of treatment, if patients received it more than 6 months prior to their cancer recurring.

3.1.3 Patients must have a biopsiable disease and at least one separate measurable lesion

3.1.4 Dose Escalation and Expansion Cohorts

3.1.4.1 ECOG performance status ≤2 (Karnofsky ≥60%, see Appendix A).

3.1.4.2 Age ≥18 years.

Because no dosing or adverse event data are currently available on the use of elimusertib (BAY 1895344) in combination with gemcitabine in patients <18 years of age, children are excluded from this study.

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

– leukocytes	≥3,000/mcL
– **hemoglobin	≥10 g/dL
– *neutrophil count	≥1,500 K/mcL
– platelets	≥100, 000 /mcL
– albumin	≥ 2.8 mg/dL
– total bilirubin	≤ 1.5 × institutional ULN
– AST(SGOT)/ALT(SGPT)	≤ 3 × institutional ULN
– creatinine clearance	≤ 1.5 × institutional ULN OR
– glomerular filtration rate (GFR)	≥50 mL/min/1.73 m^2 (see Appendix B)

* *Participants must not have received colony stimulating factors (e.g., granulocyte colony-stimulating factor, granulocyte macrophage colony stimulating factor or recombinant erythropoietin) within 3 weeks before initiation of protocol therapy.*

** *No red blood cell transfusion is allowed within 3 weeks before starting the trial*

3.1.4.4 Human immunodeficiency virus (HIV)-infected patients on effective anti-retroviral therapy with undetectable viral load within 6 months are eligible for this trial.

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

3.1.4.6 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.

3.1.4.7 Patients with **treated brain metastases** or primary brain tumors are eligible if follow-up brain imaging after central nervous system (CNS)-directed therapy shows no evidence of progression for ≥ 4 weeks after the last date of treatment are permitted, and if they are no longer taking corticosteroids for at least 4 weeks prior to beginning the protocol.

3.1.4.8 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 with permission of the principle investigator of the trial.

3.1.4.9 Patients with known history or current clinically significant 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.

3.1.4.10 The effects of elimusertib (BAY 1895344) on the developing human fetus are unknown. For this reason and because DNA-damage response inhibitor agents as well as other therapeutic

agents used in this trial are known to be teratogenic, women of child-bearing potential and men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation and for 6 months after completion ofelimusertib (BAY 1895344) administration. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately. Men treated or enrolled on this protocol must also agree to use adequate contraception prior to the study, for the duration of study participation, and 6 months after completion of elimusertib (BAY 1895344) administration.

3.1.4.11 Ability to understand and the willingness to sign a written informed consent document.

3.2 Exclusion Criteria

3.2.1 Patients cannot receive chemotherapy, targeted therapy or immunotherapy within 3 weeks of study entry.

3.2.2 Patients who have had radiotherapy within 4 weeks prior to entering the study.

3.2.3 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 and lymphopenia.

3.2.4 Participants must not have received investigational therapy administered ≤ 4 weeks or within a time interval less than at least five half-lives of the investigational agent, whichever is longer, before initiation of protocol therapy

3.2.5 Participants with known untreated brain metastases are excluded from this clinical trial.

3.2.6 History of allergic reactions attributed to compounds of similar chemical or biologic composition to elimusertib (BAY 1895344) or gemcitabine.

3.2.7 Patients receiving any medications that are substrates of CYP3A4 with a narrow

therapeutic window, or strong inhibitors/inducers of CYP3A4 are ineligible, if they cannot be transferred to alternative medication. 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. Appendix D should be presented to the patient.

3.2.8 Patients with uncontrolled intercurrent illness including but not limited to ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, clinically significant cardiac arrhythmia, uncontrolled major seizure disorder, unstable spinal cord compression, superior vena cava syndrome or psychiatric illness/social situations that would limit compliance with study requirements are excluded.

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

3.2.10 Pregnant women are excluded from this study because elimusertib (BAY 1895344) as a DNA-damage response inhibitor, and gemcitabine may have 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 elimusertib (BAY 1895344) breastfeeding should be discontinued if the mother is treated with elimusertib (BAY 1895344) and for 4 months after end of treatment. These potential risks may also apply to other agents used in this study.

3.2.11 Patients who have successfully undergone treatment for another, unrelated clinically relevant cancer, ≥ 3 years post final treatment, are eligible to participate in this study.

3.2.12 Patients cannot have received radiation to more than 25% of their hematopoietically active bone marrow. Pelvic radiation is considered to affect 25% of the haematopoietically active bone marrow, and only one prior course of pelvic radiation is allowed (Hayman *et al*., 2011).

3.2.13 Patients previously treated with an ATR inhibitor are excluded.

3.2.14 Participants who have undergone major surgery ≤ 4 weeks before initiating protocol therapy must have sufficiently recovered from adverse events caused by the procedure, as judged by the treating investigator.

3.2.15 Subjects with a gastrointestinal disorder or malabsorption that could potentially affect the absorption of the study drug are excluded.

3.2.16 Participants with a history of a clinically relevant second primary malignancy within the past 2 years are excluded. Exceptions include resected basal and squamous cell carcinomas of the skin and completely resected carcinoma *in situ* of any type.

3.2.17 Patients not able to swallow tablets.

3.2.18 For the Dose Expansion Cohort, patients who cannot safely undergo tumor biopsies are excluded.

3.3 Inclusion of Women and Minorities

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

4. REGISTRATION PROCEDURES

4.1 Investigator and Research Associate Registration with CTEP

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

RCR utilizes five person registration types.

- Investigator (IVR): MD, DO, or international equivalent,
- Non Physician Investigator (NPIVR): advanced practice providers (*e.g.*, NP or PA) or graduate level researchers (*e.g.*, PhD),
- Associate Plus (AP): clinical site staff (*e.g.*, RN or CRA) with data entry access to CTSU applications such as the Roster Update Management System (RUMS), OPEN, Rave, acting as a primary site contact, or with consenting privileges,
- 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 with a linked ID.me account (the latter required immediately for new CTEP-IAM accounts, and by July 1, 2023 for all users) is required to participate in NCI clinical trials supported by the Cancer Trials Support Unit (CTSU) and to access all CTEP and 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,
- Selection asthe treating, credit, , or drug shipment investigator or consenting person in OPEN,
- Ability to be named as the site-protocol Principal Investigator (PI) on the IRB approval, and
- Assignment of the Clinical Investigator (CI) task on the Delegation of Tasks Log (DTL).

In addition, all investigators acting as the Site-Protocol PI (Investigator listed on the IRB approval), consenting/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 (SSW) for Local Context to the CIRB using IRBManager to indicate their intent to open the study locally. The NCI CIRB's approval of the SSW is automatically communicated to the CTSU Regulatory Office, but sites are required to contact the CTSU Regulatory Office at CTSURegPref@ctsu.coccg.org to establish site preferences for applying NCI CIRB approvals across their Signatory Network. Site preferences can be set at the network or protocol level. Questions about establishing site preferences can be addressed to the CTSU Regulatory Office by emailing the email address above 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 criteria for the site to be able to have an Approved status following processing of the IRB/REB approval record:

- Have an active CTEP status,
- Have an active statusat the site on the IRB/REB approval (*applies to US and Canadian sites only*) on at least one participating organization's roster,
- If using NCI CIRB, be active on the NCI CIRB roster under the applicable CIRB Signatory Institution(s) record,
- Include the IRB number of the IRB providing approval in the Form FDA 1572 in the

RCR profile,

- List all sites on the IRB/REB approval as Practice Sites in the Form FDA 1572 in the RCR profile; and
- Have the appropriate CTEP registration type for the protocol.

Additional Requirements

Additional requirements to obtain an approved site registration status include:

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

4.1.1 Downloading Site Registration Documents

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

- Log in to the CTSU members' website (https://www.ctsu.org) using your CTEP-IAM username and password or linked ID.me account (ID.me accounts are required for all newly created CTEP-IAM accounts and by July 1, 2023 for all users,
- 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-MA036 / Dana-Farber Harvard Cancer Center, and protocol number 10403,
- Click on *Documents*, *Protocol Related Documents*, and use the *Document Type filter* and select *Site Registration*, to download and complete the forms provided. (Note: For sites under the CIRB, IRB data will load automatically to the CTSU.)

4.1.2 Protocol Specific Requirements For 10403 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).

4.1.3 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 by phone or email: 1-866-651-CTSU (2878), or CTSURegHelp@coccg.org to receive further instruction and support.

Delegation of Tasks Log (DTL)

Each site must complete a protocol-specific DTL using the DTL application 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 describes DTL task assignments, CI signature, and CTEP registration requirements , as well as include a Master Task List.

4.1.4 Checking Site Registration Status

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

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

Note: The status shown only reflects institutional compliance with site registration requirements as outlined within the protocol. It does not reflect compliance with protocol requirements for individuals participating on the protocol or the enrolling investigator's status with the 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 and linked ID.me account (ID.me accounts are required for all newly created CTEP-IAM accounts and by July 1, 2023 for all users).
- 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 Health Insurance Portability and Accountability Act (HIPAA) authorization form (if applicable).

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

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

Patient enrollment for this study will be facilitated using the Slot Reservation System in conjunction with 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 Early Phase and Experimental Clinical Trials Biospecimen Bank (EET Biobank, formerly known as the ETCTN 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 the 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 the 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 855-828-6113 or Theradex main number 609-799-7580; CTMSSupport@theradex.com.

4.4 General Guidelines

Following registration, patients should begin protocol treatment within 14 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

Dose Escalation

Time Point	Specimen	Send Specimens To:
Baseline		
	• 20 mL whole blood in Streck cfDNA tubes (mandatory)	EET Biobank
	• 5 mL blood in red-top tube, processed for serum and frozen (mandatory)	Beumer Laboratory
C1D1		
Pre-dose, 25 minutes (pre-end of infusion), 1 hr, 1.5 hr	• 5 mL blood in purple-top EDTA tube prepared with THU, processes for plasma and frozen, per time point (mandatory)	Beumer Laboratory
C1D2		
Pre-dose, 25 minutes, 1 hr, 1.5 hr, 2 hr, 4 hr, 6 hr	• 5 mL blood in purple-top EDTA tube prepared with THU, processes for plasma and frozen, per time point (mandatory)	Beumer Laboratory
C1D8		
Pre-dose, 25 min (pre-end of infusion)	• 5 mL blood in purple-top EDTA tube prepared with THU, processed for plasma and frozen, per time point (mandatory)	Beumer Laboratory

Dose Expansion

Time Point	Specimen	Send Specimens To:
Archival		
	• Formalin-fixed paraffin-embedded (FFPE) tumor rich tissue block (*strongly preferred*)[1] If a block is not available, then submit: • 1 H&E stained slide • 20-25 (10 µm) unstained uncharged slides[1] • 10-15 (4-5 µm) unstained charged slides[1] (optional)	EET Biobank
Baseline		
	• 1 tissue core, flash frozen[2,4] (mandatory) • 2 tissue cores in formalin[2] (optional, if archival tissue unavailable) • 20 mL whole blood in Streck cfDNA tubes (mandatory)	EET Biobank
	• 5 mL blood in red-top tube, processed for serum and frozen (mandatory)	Beumer Laboratory
Ovarian Cohort	• 1 tissue core in a 15 mL conical tube with DMEM (1% BSA, 1% Penn-Strep) (optional)[3]	D'Andrea Laboratory

Pancreatic Cohort	• 1 tissue core in a 15 mL conical tube with DMEM (1% Penn-Strep, 500 ng/mL Fungizone, 20 µg/mL Gentamicin) (optional)	Aguirre Laboratory
C1D1		
Pre-dose, 25 minutes (pre- end of infusion), 1 hr, 1.5 hr	• 5 mL blood in purple-top EDTA tube prepared with THU, processed for plasma and frozen, per time point (mandatory)	Beumer Laboratory
C1D2		
Pre-dose, 25 minutes, 1 hr, 1.5 hr	• 5 mL blood in purple-top EDTA tube prepared with THU, processed for plasma and frozen, per time point (mandatory)	Beumer Laboratory
C1D8		
Pre-dose	• 5 mL blood in purple-top EDTA tube prepared with THU, processed for plasma and frozen, per time point (mandatory)	Beumer Laboratory
C1D9		
	• 1 tissue core, flash frozen[2,4] (mandatory) • 2 tissue cores in formalin[2] (optional) • 20 mL whole blood in Streck cfDNA tubes (optional)	EET Biobank
Ovarian Cohort	• 1 tissue core in a 15 mL conical tube with DMEM (1% BSA, 1% Penn-Strep) (optional)[3]	D'Andrea Laboratory
Pancreatic Cohort	• 1 tissue core in a 15 mL conical tube with DMEM (1% Penn-Strep, 500 ng/mL Fungizone, 20 µg/mL Gentamicin) (optional)	Aguirre Laboratory
C3D1		

	• 20 mL whole blood in Streck cfDNA tubes (optional)	EET Biobank
Disease Progression		
	• 1 tissue core, flash frozen[2,4] (optional)	EET Biobank

	• 2 tissue cores in formalin[2] (optional) • 20 mL whole blood in Streck cfDNA tubes (optional)	
Ovarian Cohort	• 1 tissue core in a 15 mL conical tube with DMEM (1% BSA, 1% Penn-Strep) (optional)[3]	D'Andrea Laboratory
Pancreatic Cohort	• 1 tissue core in a 15 mL conical tube with DMEM (1% Penn-Strep, 500 ng/mL Fungizone, 20 µg/mL Gentamicin) (optional)	Aguirre Laboratory

[1]For archival tissue, a copy of the corresponding anatomic pathology report labeled with the Universal ID and Patient Study ID 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 – 41).

[2] For new biopsies, the Tissue Biopsy Verification Form (Appendix *F)* 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 tissue to the EET Biobank. All reports must be labeled with the Universal ID and Patient Study ID.

[3]A copy of the specimen collection log (Appendix G) should be included in the shipment to the D'Andrea Laboratory.

[4] Refer to Appendix H for detailed SOP on preparing snap frozen tumor tissue.

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

Biopsy #: 1				
Trial Time Point: Baseline				
IR Biopsy Definition: Research – No Clinical Impact (All cores from a single biopsy procedure impact research goals, but do not directly impact patient care or benefit the patient.)				
Core Priority	**Use in the Trial**	**Biomarker Name(s)**	**Tumor Content Required**	**Post-Biopsy Processing**
1	Integrated	γH2AX, pNBS1 IFA with βCATN	>15%	Flash frozen
2	Exploratory	WES, RNAseq	>15%	Formalin

3	Exploratory*	RAD51 pKAP1, pRPA32, pATR, pCHK1, SLFN11, Cyclin E, MYC, ATM expression	>15%	Formalin
4	Exploratory	Organoid generation	>5%	Fresh tissue (in appropriate cell culture media)
**The number of biomarkers assessed by IHC will depend on the amount of tissue available.*				

Biopsy #: 2				
Trial Time Point: C1D9				
IR Biopsy Definition: Research – No Clinical Impact (All cores from a single biopsy procedure impact research goals, but do not directly impact patient care or benefit the patient.)				
Core Priority	**Use in the Trial**	**Biomarker Name(s)**	**Tumor Content Required**	**Post-Biopsy Processing**
1	Integrated	γH2AX, pNBS1 IFA with βCATN	>25%	Flash frozen
2	Exploratory	RNAseq	>15%	Formalin
3	Exploratory*	RAD51 pKAP1, pRPA32, pATR, pCHK1, SLFN11, Cyclin E, MYC, ATM expression	>15%	Formalin
4	Exploratory	Organoid generation	>5%	Fresh tissue (in appropriate cell culture media)
**The number of biomarkers assessed by IHC will depend on the amount of tissue available.*				

Biopsy #: 3				
Trial Time Point: Disease Progression - **OPTIONAL**				
IR Biopsy Definition: Research – No Clinical Impact (All cores from a single biopsy procedure impact research goals, but do not directly impact patient care or benefit the patient.)				
Core Priority	**Use in the Trial**	**Biomarker Name(s)**	**Tumor Content Required**	**Post-Biopsy Processing**
1	Integral	γH2AX, pNBS1 IFA with βCATN	>25	Flash frozen
2	Exploratory	RNAseq	>15%	Formalin
3	Exploratory*	RAD51 pKAP1, pRPA32, pATR, pCHK1, SLFN11, Cyclin E, MYC, ATM expression	>15%	Formalin
4	Exploratory	Organoid generation	>5%	Fresh tissue (in appropriate cell culture media)
**The number of biomarkers assessed by IHC will depend on the amount of tissue available.*				

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 E).

5.3 Specimen Procurement Kits and Scheduling

5.3.1 Specimen Procurement Kits

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. Kits for the collection and shipment of tissue cores and blood to the EET Biobank can be ordered online via the Kit Management system: https://kits.bpc-apps.nchri.org/.

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

5.3.2 Scheduling of Specimen Collections

5.3.2.1 Scheduling of Specimen Collections for the EET Biobank

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

- Tumor tissue specimens collected during biopsy procedures and fixed in formalin must be shipped on the same day of collection.
- Tissue in formalin can be collected Monday through Wednesday and shipped overnight for arrival on Tuesday through Thursday at the EET Biobank at Nationwide Children's Hospital.
- Frozen tissue can be collected on any day but must be stored frozen and shipped to the EET Biobank 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.

5.3.2.2 Scheduling of Specimen Collections for the Beumer Laboratory at the University of Pittsburg

Blood samples will be collected at the timepoints specified in Section 5.1. Frozen plasma will be shipped overnight on either Monday, Tuesday, or Wednesday (and not before a federal or university holiday) to the Beumer Laboratory at the University of Pittsburgh.

5.3.2.3 Scheduling of Specimen Collections for the D'Andrea Laboratory (Ovarian Cancer Tissue)

- Tissue biopsy samples from the ovarian cancer cohort will be collected at the timepoints specified in Section 5.1. Samples must be placed in chilled transportation media. Samples can be stored at 4°C for no more than 24 hr. All samples must be shipped via tracked expedited overnight shipping (*e.g*. FedEx, UPS) and must be shipped to arrive the following business morning (*i.e.* must arrive Monday – Friday morning). Samples cannot be shipped to arrive on weekends or federal holidays. Notice should be given at least 2 business day in advance of sample shipment whenever possible.

5.3.2.4 Scheduling of Specimen Collections for the Aguirre Laboratory (Pancreatic Cancer Tissue)

Samples must be placed in chilled transportation media. Samples can be stored at 4°C for no more than 24hr. Samples must be shipped via tracked expedited overnight shipping (*e.g*. FedEx, UPS) and must be shipped to arrive the following business morning (*i.e.* must arrive Monday – Friday morning). Samples cannot be shipped to arrive on weekends or federal holidays. Notice should be given at least 1 business day in advance of sample shipment whenever possible.

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 without a corresponding pathology report, the radiology and operative report(s) must also be uploaded into Rave, when available. **Important: Remove any personally identifying information, including, but not limited to, the patient's name, date of birth, initials, medical record number, and patient contact information from the institutional pathology report prior to submission.**

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

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

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

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 and for PK samples the exact clock time (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):

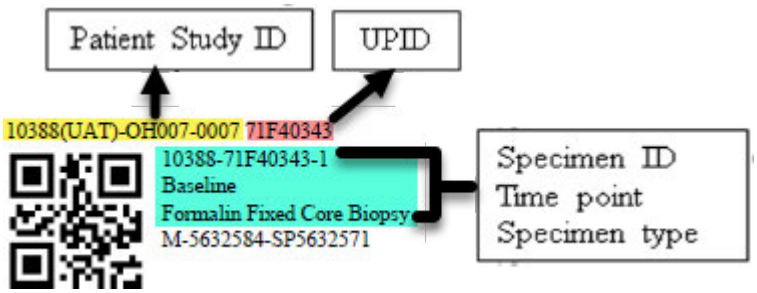
- Patient Study ID
- Universal Patient ID (UPID)
- Specimen ID (automatically generated by Rave)

- 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 user as a result of saving that form.

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



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

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

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

1. Tissue only: Primary (P), Metastatic (M), Normal (N) tissue indicated at the beginning of the specimen ID; this field is blank if not relevant (*e.g.*, for blood)
2. Block ID or blank if not relevant
3. SPID (Surgical Pathology ID) or blank if none

4. An optional alpha-numeric code that is protocol specific and is only included if the protocol requires an additional special code classification

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

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 (and time for PK samples) 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), and Surgical (or Operative) reports. Return to **Specimen Tracking Enrollment** CRF to upload any molecular report (one per specimen) and/or specimen specific pathology or related report (one per specimen) and/or Tissue Biopsy Verification form (Appendix F). Uploaded reports should have protected health information (PHI) data, like name, date of birth, mailing address, medical record number or social security number (SSN), redacted. Do not redact SPID, block number, diagnosis or relevant dates (such as collection date), and include the UPID and patient

study ID on each document (either by adding a label or handwriting).

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 6 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:

- 1 H&E stained slide
- Twenty to twenty-five (20 – 25) 10 µm unstained air-dried uncharged slides.

- Ten to fifteen (10-15) 4-5 µm unstained air-dried charged 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 Formalin-Fixed Tumor Biopsies

1. Label formalin-filled containers according to instructions in Section 5.4.2.
2. Obtain 2 16-gauge or 18-gauge core needle biopsy specimens, and place one core in each cassette.
3. Snap the cassette lids closed and place cassettes into a formalin-filled pre-labeled container as soon as possible after collection to prevent air drying. Up to two cassettes may be placed in one formalin jar.
4. Secure the container lids and 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 EET Biobank.

5.5.3 Collection of Snap-Frozen Biopsies

1. Follow step 5.3.1 to request specimen procurement kits for frozen sample collection before scheduled biopsy collection.
2. Biopsy specimens should be collected into pre-chilled 1.5mL Sarstedt, O-ring screw cap tubes (VWR, Cat#: 83009-010).
    a. Label cryovial(s) according to instructions in Section 5.4.2., prior to pre-chilling
3. It is imperative that biopsies are flash frozen within 2 minutes of collection in order to preserve key pharmacodynamic biomarkers.
4. As described in Appendix F, place the tissue in a pre-chilled cryovial and freeze the tube in liquid nitrogen or dry ice/ethanol bath. Keep frozen at -80 ºC or lower until shipment to the EET Biobank.

Sites are strongly encouraged to contact the NCLN PD Laboratory at NCI_PD_Support@mail.nih.gov to initiate training and clarify biopsy collection procedure.

5.5.2.1 Tissue collection for Organoid Cultures (Ovarian Cancer Cohort)

All samples must be labeled with the NCI protocol number (10403), study subject number, subject initials, and date of sample collection. Use labels that will not smear or fall off under cold or moist conditions.

Please fill the specimen collection log (Appendix G). Prepare two specimen labels with appropriate specimen ID. Fix one label to a 15 mL specimen collection tube. Fix the second label to the specimen collection log.

During specimen collection, three key steps to successful organoid generation will be (1)

minimizing the time between specimen collection and placement into the chilled, sterile media (2) ensuring sterility of the specimen and media during this process and (3) fast transport to the destination.

Safety
Fresh human tissues are handled under Biosafety Level 2 (BSL2) conditions.

Materials for Tissue Collection
- Ice bucket with wet ice
- Sterile disposable tweezers

- Sterile 15 mL conical polypropylene centrifuge tubes Thermo Scientific™ (Cat# 339651).
- 1% BSA (SigmaA7906)
- DMEM (ThermoFisher 10569010)
- Antibiotic: Penicillin Streptomycin (ThermoFisher 15140122)
- 0.2µ filter (ThermoFisher 450-0020)
- 2 Specimen labels
- Specimen collection log (Appendix G)

Transport Media Preparation:

In a biosafety cabinet, add 1% antibiotic and 1% BSA by weight to DMEM. For 500mL media use 5g BSA and 5mL Penn-Strep. Use a magnetic stirrer to dissolve the BSA in the media until completely dissolved (approximately 10-15 min). Once dissolved, filter sterilize using 0.2µ filter and store at 4^{o}C. Perform inside a biosafety cabinet.

Store the transport media at 4 ºC until use. Due to addition of antibiotics, an expiration date of 30 days should be applied to the media.

Sample Collection Instructions

1. All samples should be collected in 15 mL conical tubes.
2. All samples must be labeled with the NCI protocol number (10403), study subject number, subject initials, and date of collection. Use labels that will not smear or fall off under cold or moist conditions.
3. A total of one(minimum) to four (goal) core biopsy samples will be collected at each biopsy time point.
4. The maximum size for an individual piece placed in the media should be 9 mm in its longest dimension. The ratio of transport media to tissue sample should be at least 10 :1 (mL media: cm tissue)
5. Samples must be placed in the transport media immediately upon collection and stored at 4˚C until shipment.
6. In the OR or procedure suite, use sterile, disposable tweezers to transfer tissue into the chilled, sterile specimen collection tube containing transport media solution.
7. The preferred collection method is for the surgeon to place the biopsy directly into the chilled, sterile media solution.
8. After collection transfer the specimen collection tube immediately back to the ice bucket.

9. Record the time of specimen collection on the specimen collection log.
10. **Samples must be shipped within 24 hours of the biopsy procedure.**

5.5.2.2 Tissue collection for Organoid Cultures (Pancreatic Cancer Cohort)

Transport Media Preparation

The media for transportation of the cores for organoid development should be DMEM (Fisher Scientific Catalog Number: 11995065) with antibiotics. The following antibiotics should be used in the following volumes with 500 mL of DMEM:

Table 3: Organoid Transport Media

Reagent	Manufacturer	Cat. No	Type Annotation	Volume	Final Concentration
Pen/Strep (100 X)	Invitrogen	15140-163	Antibiotics	5 mL	1%
Fungizone (250 µg/mL)	Invitrogen	15290018	Antibiotics	1 mL	500 ng/mL
Gentamicin (50 mg/mL)	Life Technologies	15750-060	Antibiotics	100 µL	20 µg/mL

Sample Collection Instructions:

- All samples should be collected in 15 mL conical tubes (Fisher Scientific Catalog Number: S50712).
- All samples must be labeled with the NCI protocol number (10403), study subject number, and date of collection. Use labels that will not smear or fall off under cold or moist conditions. A total of one (minimum) to four (goal) core biopsy samples will be collected at each biopsy time point.
- Samples must be placed in the transport media immediately upon collection and stored at4˚C until shipment.
- Samples must be shipped within 24 hours of the biopsy procedure.

5.5.3 Blood Collection

5.5.3.1 Collection of Blood in Streck cfDNA Tube

1. Label one 10 mL Streck cfDNA tube according to the instructions in Section 5.4.2.
2. Collect 10 mL of blood into the pre-labeled tube and gently invert to mix. **Note:** blood must be thoroughly mixed to ensure preservation of specimen. Heparin should be avoided in pre-collection flush procedures. If therapeutic heparin dosing contamination is a possibility, then venipuncture is recommended as a first-choice collection method. If a Streck cfDNA tube immediately follows a heparin tube in the draw order, then collecting an EDTA tube as a waste tube prior to collection in the Streck Cell-Free DNA BCT is recommended.
3. **After collection, blood in Streck cfDNA tubes should never be refrigerated,** as this will compromise the specimen. Blood collected in Streck cfDNA tubes is stable at room

temperature.

5.5.3.2 Collection of Blood in Red Top Tube for Serum Processing

1. Label one 10 mL red-top tube according to the instructions in Section 5.4.2.
2. Collect 10 mL of whole blood in red-top tube.
3. Allow blood to clot upright at room temperature for at least 30 minutes (maximum 60 minutes) prior to processing. If the blood is not immediately processed after the clotting period, then tubes should be stored (after the 30-60 minutes of clotting time) at 4°C for no longer than 4 hours. Process serum from red top tubes by centrifuging for 10 minutes at 1,200 × g at room temperature.
4. **Using a clean transfer pipette,** aliquot serum into the labeled (using the label printed from the ETCTN Specimen Tracking System or following the instructions in Section 5.4.2) cryovials at an aliquot volume of 1 mL per tube. Avoid picking up red blood cells when aliquoting by keeping the pipet above the red blood cell layer and leaving a small amount of serum in the tube. Tightly secure the cap of the vials before storage. Aliquoting and freezing of serum specimens should be completed within 1 hour of centrifugation.
5. Store serum cryovials upright in a specimen box or rack in an -70°C to -90°C or colder freezer prior to delivering to laboratory. Do not allow specimens to thaw after freezing.

5.5.3.3 Collection of Blood in EDTA Tubes for Plasma Processing (elimusertib (BAY 1895344) and gemcitabine PK Biomarkers)

General
Blood samples to be obtained through a peripheral or central line blood draw. Samples should be drawn from the opposite arm if infusion is a peripheral infusion. Samples should NOT be drawn from the infusion line. On day 1 only EDTA Vacutainer tubes shall be prepared by addition of THU to prevent *ex vivo* degradation of gemcitabine, see below.

Preparation of tubes with THU
Prepare an aqueous stock solution of tetrahydrouridine (THU) at 10 mg/mL. THU can be added to Vacutainer tubes up to several days in advance without causing significant loss of vacuum (keep in fridge, no more than 1-2 weeks).

1. Using a 3/10 cc insulin syringe or other similar sized syringe with a fine needle, draw up 10 mcL of the THU solution for each 1 mL of blood to be drawn and transfer it to a Vacutainer tube by piercing the stopper.
2. Do not draw up THU solution for more than one tube at a time. You will not be able to control the volume of THU solution that leaves the needle, as it is sucked out by the vacuum.
3. Because of the fine needle, you will not lose the vacuum (apart from the volume added) in the collection tube.

Drawing and Processing

Document exact start and stop times of each infusion and exact times of blood draws.

1. Collect in a 5 mL purple top tube (*e.g.* BD vacutainer 367861 plastic 13 x 75 4 mL tube).

2. Invert the vacutainer tubes several times to mix blood with EDTA anticoagulant and immediately place on ice.
3. Processing should begin within 30 minutes of collection.
4. Samples should be centrifuged for 10 min at approximately 1000 x g in a refrigerated tabletop centrifuge so as to produce plasma.
5. The resulting plasma should be aspirated from the tubes, placed into appropriately-labeled microcentrifuge tubes (See Section 5.3.2.1), and stored at -70 °C until shipment.

5.6 Shipping Specimens from Clinical Site to the EET Biobank

5.6.1 General Shipping Information

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

5.6.1.1 Required Forms for Tissue Submissions:

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

Tissue	**Required Forms**
Archival	1. Shipping List 2. Corresponding Pathology Report
New Biopsy	1. Shipping List 2. Tissue Biopsy Verification Form (Appendix F) 3. Diagnostic Pathology Report 4. Operative and/or Radiology Report
Blood	1. Shipping List

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 tissue 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 Blood in an Ambient Shipper, for blood in Streck cfDNA tubes

1. Before packaging specimens, verify that each specimen is labeled according to the instructions in 5.4.2.1 and that the lids of all primary receptacles containing liquid are tightly sealed.
2. Prepare the SAF-T-TEMP Gel Pak for shipment. **Note:** If contents of the Pak are crunchy, place Pak in a warm water bath until gel is smooth. **Do not refrigerate, freeze, or microwave.**
3. Place the SAF-T-TEMP Pak in bottom of insulated chest. **Note:** The insulated chest must be shipped inside the provided cardboard box(es).
4. Place the blood collection tubes in zip-lock bags.
5. Next, place blood into a biohazard envelope with absorbent material. Expel as much air as possible and seal the envelope securely.
6. Place the biohazard envelope into a Tyvek envelope. Expel as much air as possible and seal securely.
7. Place packaged blood collection tube(s) and a copy of the shipping manifest from the Sample Tracking System on top of SAF-T-TEMP Pak.
8. Place the lid on the insulated chest.
9. Close the outer flaps of the shipping box and tape shut.
10. Attach a shipping label to the top of the shipping container.
11. Attach an Exempt Human Specimen sticker to the side of the box.
12. Ship specimens via overnight courier to the address below. FedEx Priority Overnight is strongly recommended to prevent delays in package receipt.

5.6.2.3 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 <u>both</u> 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 5.4.2and 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 formalin-fixed and fresh blood specimens the same day of specimen collection. Do not ship specimens the day before a holiday.

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

to the EET Biobank.

5.6.4 Contact Information for Assistance

For all queries, please use the contact information below:

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

5.7 Shipping of Specimens from Clinical Site to Other Laboratories

5.7.1 Shipping of Specimens to Jan Beumer University of Pittsburg (PK-plasma and PK-serum)

5.7.1.1 Specimen Shipping Instructions

Preparing the shipment

1. Samples should be stored in cardboard boxes (5 1/8" x 5 1/8" x 2", L x W x H).
2. Please organize the samples by Patient and Time point in the box.
3. Do not store in plastic bags (they break on dry-ice and labels will detach).
4. A copy of each of the pharmacokinetic sample collection forms for the respective patients should be included with each shipment. To prevent problems with illegible writing on tubes, consider numbering them and numbering samples on the sample sheet.

*Note the study number, PI, and the drugs used/to be measured.
*A name, phone number, and email address should be included with the samples so that receipt can be acknowledged.

5. Please notify the lab by email (PITT-PK@UPMC.EDU), telephone (412-623-3248) or fax (412-623-1212) at least 24 hours prior to shipment.

Regulations

Shipment of samples must comply with appropriate regulations as specified by the carrier. At a minimum, all samples must be packaged within two containers with absorbent material between containers to control any spill or leakage. The outer container must be puncture-resistant (*e.g.* cardboard mailing tube, corrugated cardboard box). A biohazard sticker must be affixed to both the inner and outer containers.

5.7.1.2 Shipping Address

All samples should be shipped via overnight express courier in insulated containers with enough dry ice to maintain the samples in a frozen state. All specimens are to be shipped on either Monday, Tuesday, or Wednesday, and not before federal or university holidays.

Cancer Pharmacokinetics and Pharmacodynamics Facility
UPMC Hillman Cancer Center
Room G27 Hillman Research Laboratories

5117 Centre Avenue
Pittsburgh, PA 15213

5.7.1.3 Contact Information for Assistance

Lab phone: 412-623-3248
Lab fax: 412-623-1212
PK Lab email: PITT-PK@upmc.edu
PK director email: beumerjh@upmc.edu

5.7.2 Shipping of Specimens to the D'Andrea Laboratory at the Dana-Farber Cancer Institute

5.7.2.1 Materials for shipping

- Two temperature-controlled gel packs.
- Absorbent material
- Bubble wrap
- Parafilm
- Necessary instructions, paperwork
- Two zip lock bags
- Styrofoam box
- Shipping cardboard box

5.7.2.2 Specimen Shipping Instructions

Preparing the Shipment

Samples must be shipped within 24 hours of the biopsy procedure.

Samples should be placed in a primary receptacle (*e.g.* insulated cooler/Styrofoam container), then into a secondary receptacle (*e.g.* sturdy cardboard box). These primary and secondary containers are available commercially as combination packaging. A shipping kit (Fisher Scientific Catalog Number: 22-13-400) can be used for shipment. The package contents are placed in this order:

1. Seal the sample tube with parafilm then wrap the samples in enough absorbent material to absorb at least three times the contents should leakage occur.
2. Place the wrapped samples in a plastic bag and seal (heat seal or zip lock).
3. The packing process should go quickly – do not leave contents at room temperature for a prolonged time.
4. Leave the cold packs in the freezer until ready to package the specimen for shipping. Ensure rapid transport of specimens to the laboratory for preparation for shipping.
5. Place a cold refrigerant pack at the bottom of the box.
6. Place the sealed bag on top of the refrigerant pack (do NOT use wet ice).

7. Place a cold refrigerant pack on top of the sample.
8. Add a sheet of cardboard onto the top of the primary receptacle.
9. If there is room remaining, add filler material to avoid content movement during transport.
10. The primary container should be taped shut and then placed in the secondary cardboard container.
11. Complete a specimen collection log. A copy of an example form is in Appendix G. If possible, also send this electronically via email.
12. Seal the specimen collection log (s) into a protected plastic bag (zip lock).
13. Place the plastic bag containing the specimen collection log (s) on top of the secured primary container lid (insulted cooler/styrofoam container) so that it is immediately accessible upon opening the cardboard box.
14. Tape the shipping box securely closed. Use tape that is resistant to moisture and cold.
15. Label the box exterior in accordance with the applicable DOT CFR / IATA Regulations.
16. Complete a shipping label and attach it to the outside of the box.

Advanced notice of incoming shipments must be given to:

Arindam_Bose@dfci.harvard.edu and Kelsey_McQueen@dfci.harvard.edu

Notice should be given at least 2 business day in advance of sample shipment whenever possible.

Samples must be shipped via tracked expedited overnight shipping (e.g. FedEx, UPS) and must be shipped to arrive the following business morning (i.e. must arrive Monday – Friday morning). Samples cannot be shipped to arrive on weekends or federal holidays.

- For DF/HCC sites beyond DFCI, samples can be same-day couriered the day of the procedure and should be packed per the shipping instructions.
- For DFCI, samples will be collected by the DCBC team and brought directly to the Aguirre Laboratory.
- For sites external to DF/HCC, or if same-day courier is not available for DF/HCC sites beyond DFCI, note that biopsy procedures **must** be scheduled to occur on Sundays – Thursdays so that samples will arrive to the Aguirre Laboratory during regular business hours Mondays – Fridays. Note that biopsies must also **NOT** be scheduled to occur the day prior to a federal holiday.

5.7.2.3 Shipping Address

Arindam Bose
77 Avenue Louis Pasteur, HM-0331E, building HM floor 3 room 31E, Boston, MA 02115.
Arindam_Bose@dfci.harvard.edu
1-857-215-2209

5.7.2.4 Contact Information for Assistance

Arindam_Bose@dfci.harvard.edu and Kelsey_McQueen@dfci.harvard.edu

5.7.3 Shipping of Specimens to the Aguirre Laboratory at the Dana-Farber Cancer Institute

5.7.3.1 Specimen Shipping Instructions

Samples must be shipped within 24 hours of the biopsy procedure.

Samples should be placed in a primary receptacle (*e.g*. insulated cooler/Styrofoam container), then into a secondary receptacle (*e.g*. sturdy cardboard box). These primary and secondary containers are available commercially as combination packaging. A shipping kit (Fisher Scientific Catalog Number: 22-13-400) can be used for shipment. The package contents are placed in this order:

1. Wrap the samples in enough absorbent material to absorb at least three times the contents should leakage occur.
2. Place the wrapped samples in a plastic bag and seal (heat seal or zip lock).
3. Place a cold refrigerant pack at the bottom of the box.
4. Place the sealed bag on top of the refrigerant pack (do NOT use wet ice).
5. Place a cold refrigerant pack on top of the sample.
6. Add a sheet of cardboard onto the top of the primary receptacle.
7. If there is room remaining, add filler material to avoid content movement during transport.
8. The primary container should be taped shut and then placed in the secondary cardboard container.
9. Complete a sample inventory form. A copy of an example form is below. If possible also send this electronically via email.
10. Seal the sample inventory form(s) into a protected plastic bag (zip lock).
11. Place the plastic bag containing the sample inventory form(s) on top of the secured primary container lid (insulted cooler/Styrofoam container) so that it is immediately accessible upon opening the cardboard box.
12. Tape the shipping box securely closed. Use tape that is resistant to moisture and cold.
13. Label the box exterior in accordance with the applicable DOT CFR / IATA Regulations.
14. Complete a shipping label and attach it to the outside of the box.

Advanced notice of incoming shipments must be given to:

Lauren Brais (LaurenK_Brais@DFCI.HARVARD.EDU; 617-632-6758)
Emma Coleman (Emmac_coleman@dfci.harvard.edu; 617-632-6301)
Joshua Remland (Joshua_Remland@DFCI.HARVARD.EDU; 617-632-2476)

Notice should be given at least 1 business day in advance of sample shipment whenever possible.

Samples must be shipped via tracked expedited overnight shipping (e.g. FedEx, UPS) and must be shipped to arrive the following business morning (i.e. must arrive Monday – Friday morning). Samples cannot be shipped to arrive on weekends or federal holidays.

For DF/HCC sites beyond DFCI, samples can be same-day couriered the day of the procedure and should be packed per the shipping instructions.

For DFCI, samples will be collected by the DCBC team and brought directly to the Aguirre Laboratory.

For sites external to DF/HCC, or if same-day courier is not available for DF/HCC sites beyond DFCI, note that biopsy procedures must be scheduled to occur on Sundays – Thursdays so that samples will arrive to the Aguirre Laboratory during regular business hours Mondays – Fridays. Note that biopsies must also NOT be scheduled to occur the day prior to a federal holiday.

5.7.3.2 Shipping Address:

Lauren Brais
Dana-Farber Cancer Institute
450 Brookline Ave., D-1220
Boston, MA 02215

5.7.3.3 Contact Information for Assistance

Lauren Brais (LaurenK_Brais@DFCI.HARVARD.EDU; 617-632-6758)
Emma Coleman (Emmac_coleman@dfci.harvard.edu; 617-632-6301)
Joshua Remland (Joshua_Remland@DFCI.HARVARD.EDU; 617-632-2476)

5.8 Biomarker Plan

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

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	γH2AX, ρNBS1 IFA with βCATN	Multiplex IFA CLIA: N	Integrated Analyze changes in γH2AX and ρNBS1 in response to the drug combination	Flash frozen tumor	**Dose Expansion:** Baseline prior to study treatment C1D9 Disease progression	M M O	NCLN PD Assay Laboratory at MD Anderson Kate Ferry-Galow, Ph.D. ferrygalowkv@mail.nih.gov
2	WES	NGS CLIA: N	Exploratory Evaluate genomic markers of response and resistance	DNA from FFPE tumor	**Dose Expansion:** Archival or baseline prior to study treatment Disease progression	O	NCLN Genomics Laboratory or MoCha, Frederick National Laboratory for Cancer Research (FNLCR) Mickey Williams, Ph.D. mickey.williams@nih.gov
3	RNAseq	NGS CLIA: N	Exploratory Evaluate genomic markers of response and resistance	RNA from FFPE tumor	**Dose Expansion:** Archival or baseline prior to study treatment C1D9 Disease progression	O	NCLN Genomics Laboratory or MoCha, Frederick National Laboratory for Cancer Research (FNLCR) Mickey Williams, Ph.D. mickey.williams@nih.gov
4	ATM Expression	IHC (Clone Y170, Abcam) CLIA: N	Exploratory To evaluate the relationship between ATM protein expression and response to treatment	FFPE Tumor Block Required	**Dose Expansion:** Archival or baseline prior to study treatment	O	Ventana

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
5	RAD51	IHC CLIA: N	Exploratory To assess whether tumor samples are HR proficient	Unstained slides from FFPE tumor	**Dose Expansion:** Archival or baseline prior to study treatment C1D9 Disease progression	O	Center for DNA Damage and Repair, Dana-Farber Cancer Institute (DFCI) Geoffrey Shapiro, M.D., Ph.D. Geoffrey_Shapiro@dfci.harvard.edu
6	pKAP1, pRPA32, pATR, pCHK1, SLFN11, Cyclin E, MYC	IHC CLIA: N	Exploratory Assess markers of ATRi sensitivity, DNA damage, and replicative stress	Unstained slides from FFPE Tumor	**Dose Expansion:** Archival or baseline prior to study treatment C1D9 Disease progression	O	Center for DNA Damage and Repair, Dana-Farber Cancer Institute (DFCI) Geoffrey Shapiro, M.D., Ph.D. Geoffrey_Shapiro@dfci.harvard.edu
7	Organoid cultures	Organoids CLIA: N	Exploratory Drug sensitivity assays Drug sensitivity testing will enable assessment of resistance to DNA repair inhibitor combination	Organoid cultures from tumor Biopsy	**Dose Expansion:** Baseline prior to study treatment C1D9 Disease progression	O	DFCI Hale Center for Pancreatic Cancer Research (pancreatic samples) Andrew Aguirre, M.D., Ph.D. andrew_aguirre@dfci.harvard.edu DFCI Center for DNA Damage and Repair (ovarian samples) Alan D'Andrea, M.D Alan_dandrea@dfci.harvard.edu
8	Replication Fork Stability	DNA fiber assay CLIA: N	Exploratory Enable evaluation of replicative stress	Organoid cultures from tumor Biopsy	**Dose Expansion:** Baseline prior to study treatment C1D9 Disease progression	O	Center for DNA Damage and Repair, Dana-Farber Cancer Institute (DFCI) Geoffrey Shapiro, M.D., Ph.D. Geoffrey_Shapiro@dfci.harvard.edu

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
Blood-based Biomarkers							
1	PK (Plasma)	LC/MS CLIA: N	Integrated Plasma pharmacokinetics of gemcitabine and elimusertib (BAY1895344) will be defined in all participants enrolled in the dose escalation and dose expansion portions of the trial to assess potential drug interactions	Plasma from EDTA prepared with THU	**Dose Escalation** C1D1: pre-dose, 25 minutes (pre-end of infusion), 1 hr, 1.5 hr, C1D2: pre-dose, 25 minutes, 1 hr, 1.5 hr, 2 hr, 4 hr, 6 hr C1D8: pre-dose, 25 minutes (pre-end of infusion) **Dose Expansion** C1D1: pre-dose, 25 minutes (pre-end of infusion), 1 hr, 1.5 hr C1D2: pre-dose, 25 minutes, 1 hr, 1.5 hr C1D8: pre-dose	M	Cancer Pharmacokinetics and Pharmacodynamics Facility, UPMC Hillman Cancer Center Jan Beumer beumerjh@upmc.edu
2	PK (Serum)	LC/MS CLIA: N	Integrated To monitor drug levels	Serum from red-top tube	**Dose Escalation and Expansion:** Baseline prior to study treatment	M	Cancer Pharmacokinetics and Pharmacodynamics Facility, UPMC Hillman Cancer Center Jan Beumer beumerjh@upmc.edu
3	ctDNA sequencing	NGS CLIA: N	Integrated Analysis of DNA damage response-related genes	Plasma from blood in Streck cfDNA tube	**Dose Escalation and Dose Expansion:** Baseline prior to study treatment **Dose Expansion:** C1D9 C3D1 Disease progression	M-Baseline O – C1D9 C3D1 Disease progression	NCLN Genomics Laboratory or MoCha, Frederick National Laboratory for Cancer Research (FNLCR) Mickey Williams, Ph.D. mickey.williams@nih.gov

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
4	WES	NGS CLIA: N	Exploratory Germline control	DNA from blood in Streck cfDNA tube	Baseline prior to study treatment	M (if tissue collected for WES)	NCLN Genomics Laboratory or MoCha, Frederick National Laboratory for Cancer Research (FNLCR) Mickey Williams, Ph.D. mickey.williams@nih.gov

5.9 Integrated Correlative Studies

5.9.1 γH2AX, ρNBS1 IFA with βCATN

5.9.1.1 Specimen(s) Receipt and Processing at the EET Biobank

Frozen cores will be sent to the Biorepository and stored in a liquid nitrogen vapor phase freezer at until shipment to the NCLN PD Laboratory for analysis.

Frozen cores will be sent to the EET Biobank and stored at -80C until shipment to the NCLN PD Laboratory for analysis.

5.9.1.2 Site(s) Performing Correlative Study

This assay will be performed at the NCLN PD Assay Laboratory at MD Anderson under the guidance of Kate Ferry-Galow, Ph.D.

5.9.1.3 Shipment of Specimens from the EET Biobank to Site Performing Correlative Study

Specimens will be shipped from the EET Biobank to:

NCLN PD Assay Laboratory at MD Anderson
Attention: Taiwo Famobio
The University of Texas MD Anderson Cancer Center
TQL-CTLU, Rm G1.3598
1515 Holcombe Blvd.
Houston, TX 77030
Tel: 713-792-5276

5.9.1.4 Contact Information for Notification of Specimen Shipment

NCLN_IFA@mdanderson.org

5.9.2 PK (Plasma and Serum)

5.9.2.1 Specimen(s) Receipt and Processing at the University of Pittsburg

Frozen Plasma and Serum aliquots will be received at the Beumer laboratory for processing and storage.

5.9.2.2 Site Performing the Correlative Studies

This assay will be performed at Dr. Jan Beumer's laboratory at the University of Pittsburgh.

The LC-MS/MS assay for elimusertib (BAY 1895344) is being developed at the UPMC Hillman Cancer

Center, Cancer Pharmacokinetics and Pharmacodynamics Facility. No published assay currently exists.

The LC-MS/MS assays to quantitate gemcitabine and its metabolite dFdU are currently implemented in the UPMC Hillman Cancer Center, Cancer Pharmacokinetics and Pharmacodynamics Facility (Stroller *et. al*, 2017).

5.9.2.3 Contact Information for Notification of Specimen Shipment

See section 5.7.1

5.10 Exploratory Correlative Studies

5.10.1 WES

5.10.1.1 Specimen Receipt and Processing at the EET Biobank

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 when needed. Following macrodissection, tumor tissue from unstained slides will be scraped for co-extraction of DNA and RNA. DNA will be extracted from the whole blood collected in the cfDNA Streck tube at Baseline. The nucleic acids will be analyzed to determine concentration and quality. Aliquots of DNA will be shipped to the central sequencing laboratory for analysis.

5.10.1.2 Site Performing Correlative Study

WES will be conducted in the NCLN Genomics Laboratory or MoCha, Frederick National Laboratory for Cancer Research (FNLCR) under the leadership of MickeyWilliams, Ph.D.

5.10.1.3 Shipment of Specimens from the EET Biobank to Site Performing Correlative Study

Specimens will be shipped from the EET Biobank to one of the laboratories designated in Section 5.10.1.2.

5.10.1.4 Contact Information for Notification of Specimen Shipment

Thomas Forbes (NCLNGenomicsReceiving@nih.gov)

5.10.2 RNAseq

5.10.2.1 Specimen Receipt and Processing at the EET Biobank

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 when needed. Following macrodissection, tumor tissue from unstained slides will be scraped for co-extraction of DNA and RNA. DNA will be extracted from the whole blood collected in the cfDNA Streck tube at Baseline. The nucleic acids will be analyzed to

determine concentration and quality. Aliquots of DNA will be shipped to the central sequencing laboratory for analysis.

5.10.2.2 Site Performing Correlative Study

RNA Seq will be conducted in the NCLN Genomics Laboratory or MoCha, Frederick National Laboratory for Cancer Research (FNLCR) under the leadership of MickeyWilliams, Ph.D.

5.10.2.3 Shipment of Specimens from the EET Biobank to Site Performing Correlative Study

Specimens will be shipped from the EET Biobank to one of the laboratories designated in Section 5.10.2.2.

5.10.2.4 Contact Information for Notification of Specimen Shipment

Thomas Forbes (NCLNGenomicsReceiving@nih.gov)

5.10.3 RAD51

5.10.3.1 Specimen Receipt and Processing at the EET Biobank

Tumor tissue received in formalin will be paraffin-embedded. All 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. Unstained slides will be shipped for this assay.

5.10.3.2 Site Performing the Correlative Study

RAD51 assay will be conducted in the Center for DNA damage and repair, Dana-Farber Cancer Institute, under the leadership of Geoffrey Shapiro, M.D., Ph.D.

5.10.3.3 Shipment of Specimens from the EET Biobank to Site Performing Correlative Study

Specimens will be shipped from the EET Biobank to:

Shipping Information:

Center for DNA Damage and Repair
ATTN: Bose Kochupurakkal
77 Avenue Louis Pasteur, HM-0331E, building HM floor 3 room 31E, Boston, MA 02115.
bose_kochupurakkal@dfci.harvard.edu
1-857-215-2209

5.10.3.4 Contact Information for Notification of Specimen Shipment

Geoffrey Shapiro, M.D., Ph.D.
Geoffrey_Shapiro@dfci.harvard.edu

5.10.4 pKAP1, pRPA32, pATR, pCHK1, SLFN11, Cyclin E, MYC

5.10.4.1 Specimen Receipt and Processing at the EET Biobank

Tumor tissue received in formalin will be paraffin-embedded. All 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. Unstained slides will be shipped for this assay

5.10.4.2 Site Performing the Correlative Study

pKAP1, pRPA32, pATR, pCHK1, SLFN11, Cyclin E, MYC, - will be conducted in the Center for DNA damage and repair, Dana-Farber Cancer Institute, under the leadership of Geoffrey Shapiro, M.D., Ph.D.

5.10.4.3 Shipment of Specimens from the EET Biobank to Site Performing Correlative Study

Specimens will be shipped from the EET Biobank to:

Shipping Information:

Center for DNA Damage and Repair
ATTN: Bose Kochupurakkal
77 Avenue Louis Pasteur, HM-0331E, building HM floor 3 room 31E, Boston, MA 02115.
bose_kochupurakkal@dfci.harvard.edu
1-857-215-2209

5.10.4.4 Contact Information for Notification of Specimen Shipment

Geoffrey Shapiro, M.D., Ph.D.
Geoffrey_Shapiro@dfci.harvard.edu

5.10.5 Organoid cultures

5.10.5.1 Specimen Receipt and Processing at the Aguirre and D'Andrea Laboratories at the Dana-Faber Cancer Institute

Specimens in media will be received and processed per site SOPs.

5.10.5.2 Site Performing the Correlative Studies

Pancreatic cancer organoid cultures will be developed at the DFCI under the leadership of

Andrew Aguirre M.D., Ph.D. Ovarian cancer organoid cultures will be developed at the DFCI under the leadership of Alan D'Andrea M.D.

5.10.5.3 Contact Information for Notification of Specimen Shipment

See Sections 5.7.2 (ovarian cohort) and 5.7.3 (pancreatic cohort).

5.10.6 Replication Fork Stability Assay

5.10.6.1 Specimen Receipt and Processing at the Aguirre and D'Andrea Laboratories at the Dana-Faber Cancer Institute

Specimens in media will be received and processed into organoid cultures by the Aguirre Laboratory for pancreatic cancer culture or the D'Andrea Laboratory for ovarian cancer samples. The organoid cultures will be transferred to the laboratory of Geoffrey Shapiro for analysis.

5.10.6.2 Site performing the Correlative Study

The Replication Fork Stability ass ay will be conducted in the Center for DNA damage and repair, Dana-Farber Cancer Institute, under the leadership of Geoffrey Shapiro, M.D., Ph.D.

5.10.6.3 Shipment of Specimens from the EET Biobank to Site Performing Correlative Study

Specimens will be shipped from the EET Biobank to:

Shipping Information:

Center for DNA Damage and Repair
ATTN: Bose Kochupurakkal
77 Avenue Louis Pasteur, HM-0331E, building HM floor 3 room 31E, Boston, MA 02115.
bose_kochupurakkal@dfci.harvard.edu
1-857-215-2209

5.10.6.4 Contact Information for Notification of Specimen Shipment

Geoffrey Shapiro, M.D., Ph.D.
Geoffrey_Shapiro@dfci.harvard.edu

5.10.7 ctDNA sequencing

5.10.7.1 Specimen(s) Receipt and Processing at the EET Biobank

Whole blood collected in Streck cf DNA tubes will be centrifuged to separate plasma. Following plasmaprocessing, DNA will be processed from blood at Baseline. Buffycoat will be processed from blood at all other time points. Plasma and buffy coat aliquots will be stored in a

-80°C freezer.

5.10.7.2 Site performing the Correlative Study

ctDNA sequencing will be conducted in the NCLN Genomics Laboratory or MoCha, Frederick National Laboratory for Cancer Research (FNLCR) under the leadership ofMickey Williams, Ph.D.

5.10.7.3 Shipment of Specimens from the EET Biobank to Site Performing Correlative Study

Specimens will be shipped from the EET Biobank to one of the laboratories designated in Section 5.10.7.2.

5.10.7.4 Contact Information for Notification of Specimen Shipment

Thomas Forbes (NCLNGenomicsReceiving@nih.gov)

5.10.8 ATM assay

5.10.8.1 Specimen(s) Receipt and Processing at EET Biobank

Tumor tissue received in formalin will be paraffin-embedded. All 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. Unstained slides will be shipped for this assay

5.10.8.2 Site performing the Correlative Study

ATM assay will be conducted by Ventana laboratory.

5.10.8.3 Contact Information for Notification of Specimen Shipment

TBD

6. TREATMENT PLAN

6.1 Agent Administration

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

Dose Escalation

Dose Level	Gemcitabine Dose	Cycle Length
-1	100mg/m2 (Day 1 and 8)	21 days
1	175mg/m2 (Day 1 and 8)	21 days
2	250mg/m2 (Day 1 and 8)	21 days
3	350mg/m2 (Day 1 and 8)	21 days
4	500mg/m2 (Day 1 and 8)	21 days

Dose Level	Elimusertib (BAY1895344)	Cycle Length
-2	10mg once daily (Days 2-3 and Days 9-10)	21 days
-1	20mg once daily (Days 2-3 and Days 9-10)	21 days
1	20mg twice daily (Days 2-3 and Days 9-10)	21 days
2	30mg twice daily (Days 2-3 and Days 9-10)	21 days
3	40mg twice daily (Days 2-3 and Days 9-10)	21 days

Regimen Description – Dose Escalation					
Agent	**Premedications ; Precautions**	**Dose**	**Route**	**Schedule**	**Cycle Length**
Elimusertib (BAY 1895344)	Dexamethasone 4 mg po QD on days of elimusertib (BAY1895344) dosing**	Per dose level	PO, QD or BID	D2-3, D9-10	21 days
Gemcitabine	Long-acting 5-HT3 antagonists* Other premedications per local hospital standards	Per dose level	IV	Days 1 and 8 of each cycle	

Dose Expansion

Agent	Dose	Route	Schedule	Cycle Length
Elimusertib (BAY 1895344)	RP2D	PO	QD or BID, D2-3, D9-10	21 days
Gemcitabine	RP2D	IV	D1, 8	21 days
RP2D = Recommended Phase 2 Dose, IV = Intravenous, PO = Orally, BID = Twice daily				

Regimen Description – Dose Expansion					
Agent	**Premedications ** ; Precautions**	**Dose**	**Route**	**Schedule**	**Cycle Length**

Elimusertib (BAY 1895344)	Dexamethasone 4 mg po QDon days of Elimusertib (BAY1895344) Dosing**	RP2D	PO, QD or BID	D 2-3, D9-10	21 days
Gemcitabine	Long-acting 5-HT3 antagonists* Other premedications per local hospital standards	RP2D	IV	Days 1 and 8 of each cycle	
**Long acting 5-HT3 antagonist (such as palonosetron) can be substituted by local hospital standard or investigator discretion* ***After cycle 2, premedications and Dexamethasone dosing can be stopped or modified based on investigator discretion* ****Doses as appropriate for assigned dose level.*					

Note: The patient will be requested to maintain a medication diary of each dose of medication (elimusertib (BAY 1895344)). The medication diary will be returned to clinic staff at the end of each course.

6.1.1 Elimusertib (BAY 1895344)

Elimusertib (BAY 1895344)tablets should be taken at least one (1) hour before or two (2) hours after a meal with approximately 240 mL of water.

If a subject vomits after taking study medication, the subject should be instructed not to retake the dose and should take the next dose as originally scheduled.

On days when elimusertib (BAY 1895344) is taken, it should be administered either once a day or every 12 hours (+/- 3 hours). Elimusertib (BAY1895344) cannot be crushed, chewed, or dissolved in water.

Missed doses of Elimusertib (BAY1895344) cannot be made up. Vomited doses should not be retaken.

6.1.2 Gemcitabine

Gemcitabine is administered intravenously at a dose of 100, 175, 250, 350 or 500 mg/m^2 over approximately 30 minutes on Days 1 and 8 of each 21-day cycle. Gemcitabine should be administered per institutional policy/package label.

Infusion time should not exceed 60 minutes. In clinical trials evaluating the maximum tolerated dose of gemcitabine, prolongation of the infusion time beyond 60 minutes or more frequent than weekly dosing resulted in an increased incidence of clinically significant hypotension, severe flu-like symptoms, myelosuppression, and asthenia.

Prior to each dose of gemcitabine, obtain a complete blood count (CBC) with a differential and a platelet count. Dose modifications should proceed as recommended in section 7.

Myelosuppression manifested by neutropenia, thrombocytopenia, and anemia occurs with gemcitabine as a single agent, and the risks are increased when gemcitabine is combined with other cytotoxic drugs.

6.2 Definition of Dose-Limiting Toxicity

A DLT is defined as any of the following:

1. Grade 4 thrombocytopenia (platelet count < 25,000/mm^3) or Grade 3 thrombocytopenia (platelet count < 50,000/mm^3) associated with bleeding, and/or requirement for transfusion and/or lasting > 7 days.
2. Febrile neutropenia (ANC < 1.0×10^9/L and fever ≥ 38.5°C). Grade 4 neutropenia > 7 days.
3. Any non-hematologic toxicity equal to or above grade 3 considered by the investigator to be associated with the study drugs. This excludes alopecia and includes only diarrhea, nausea or vomiting when optimal prophylactic measures have been prescribed. Grade 3 hyponatremia, hypokalemia, hypomagnesemia and hypophosphatemia are considered dose limiting only if they continue for ≥ 5 days despite maximal medical management. In addition, consistent with other ATR inhibitor trials, any grade 3 elevation of AST, ALT, alkaline phosphatase (ALP) (of liver origin), or gamma-glutamyl transferase (GGT) lasting ≤5 days is not considered a DLT (Middleton et al., 2021).
4. A delay in therapy of >2 weeks due to treatment-associated toxicity will be considered dose limiting.
5. Any patient unable to complete the full 21-day cycle due to toxicity, even if not under the DLT definitions, is considered a DLT and does not need to be replaced.

Patients who experience a DLT are allowed to continue on trial after discussion with the principal investigator.

In cycle 1 of the dose escalation, dose modification of elimusertib ATR (BAY 1895344) and/or gemcitabine is only after discussion with the principal investigator.

Subjects will be considered evaluable for DLT if they complete the first 21-day cycle of study drug treatment without a DLT or experience a DLT at any time during the first 21-day treatment cycle; patients who are not evaluable will be replaced.

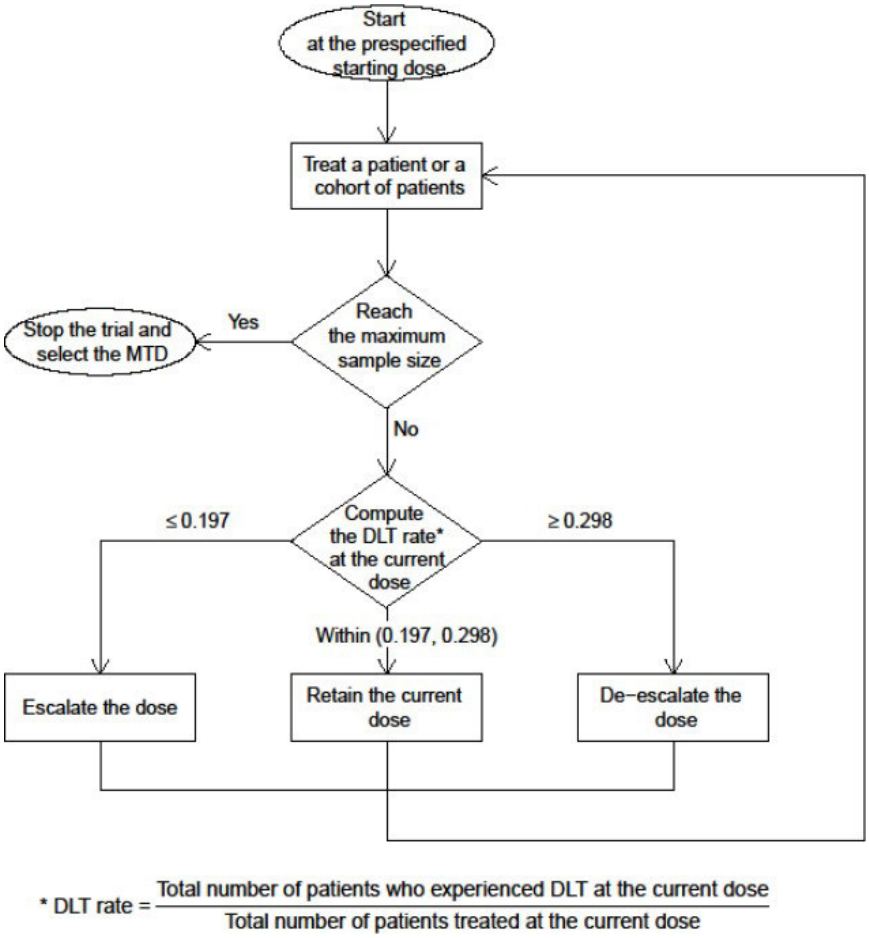
Only DLTs that occur during the first cycle will be considered for the definition of MTD. However, all patients who receive a dose of drug should be evaluable for toxicity.

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

6.3 Bayesian Dose Escalation

Dose escalation will be conducted according to a Bayesian design. The targeted dose limiting toxicity (DLT) rate is 25% (under- and overdosing boundaries of 15% and 35%, respectively). The maximum sample size of the dose escalation is 36. We will enroll and treat patients in cohorts of size 3. A maximum of 9 patients can be treated at any dose level. If the observed DLT rate at the current dose is ≤0.197, we will escalate; if the observed DLT rate at the current dose is ≥0.298, we will de-escalate; otherwise, we will stay at the current dose. We will determine maximum tolerated doses (MTDs) based on DLT rate. The design will start dosing at Gemcitabine 175mg/m2 (Day 1 and 8) and 20mg elimusertib (BAY 1895344) ATR (Days 2 -3 and Days 9-10). When more than one dose level is open in a Zone, patients will be assigned by randomization to the cohort corresponding to one of the open dose levels.

Figure 4



The trial design is illustrated in Figure 4 and described as follows:

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

2. To assign a dose to the next cohort of patients, candidate doses levels for "Stay", "Escalation", "De-escalation", "Reconsider" are listed according to the rule displayed in Table 4. The next action is decided at the Investigator Meeting

Table 4: Number of Patients with DLT for Dose Escalation Decisions

	Number of Evaluable Patients						
	3	**4**	**5**	**6**	**7**	**8**	**9**
Escalate allowed	0	0	0	0-1	0-1	0-1	0-1-2
Stay		1	1		2	2	
De-escalate and revisit allowed	1			2		3	
De-escalate and revisit not allowed (eliminate)	2-3	≥2	≥2	≥3	≥3	≥4	≥3

When using Table 4, please note the following:

a. "Eliminate" means eliminate the current and higher doses (i.e., with a higher level of both drugs at the same time) from the trial to prevent treating any future patients at these doses because they are overly toxic.

b. "Escalation" to a dose level is allowed when both lower doses (i.e., the ones with a lower level of one of the two considered drugs) suggest escalation according to Table 4. When Gemcitabine is 175mg/m2, it is sufficient that lower doses of Gemcitabine have not been explored. Similarly, when elimusertib (BAY 1895344) ATR is at 20mg it is sufficient that lower doses of elimusertib (BAY 1895344) ATR have not been explored. If there are no lower doses in Table 5 this condition is not necessary.

c. "De-escalation" to a dose level is allowed when: (i) both lower doses (i.e., the ones with a lower level of one of the two considered drugs) suggest escalation according to Table 4; (ii) when a higher dose level (respect to either one or both drugs) suggest "De-escalate" according to Table 4; (iii) the current dose has already been explored and classified as "Stay" or "Escalate" according to Table 4.

c. When we eliminate a dose, automatically de-escalate the dose to a lower level, decreasing the dose of at least one of drugs. The considered dose level will be decided at the investigator meeting. When the lowest dose is eliminated, stop the trial for safety. In this case, no dose should be selected as the MTD.

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

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

f. If the current dose is the highest dose and the rule indicates dose escalation, treat the new patients at the highest dose.

g. No more than two dose levels can be opened at one time. If the Bayesian design indicates that more than two dose levels can be opened, this will be discussed at the investigator meeting and investigators will decide which two dose levels to use for the next cohort of patients. If a situation arises where two contiguous levels can be opened, for safety purposes the lower dose needs to be opened.

• Repeat step 2 until the maximum sample size of 36 is reached or stop the trial early when one of the following two conditions is satisfied:

- The number of patients who experienced DLTs at the lowest dose level reaches the stopping boundaries listed in Table 5. In this case, no dose should be selected as the MTD.
- The number of patients treated at the current dose = 9, and no other action are allowed.

At the completion of the escalation/de-escalation study, we will estimate the toxicity probabilities of all dose combinations based on the toxicity data collected from all the zones using isotonic regression. We will select the recommended dose as the dose combination that has the estimate of toxicity probability closest to the target DLT rate of 25%.

Table 5 and **Figure 5** show the conduct of the dose escalation design, with 6 Zones. Dose Level (DL) 1 constitutes Zone 1. DL2 and DL5 are in Zone 2. DL3, DL6 and DL9 are in Zone 3. DL4, DL7 and DL10 are in Zone 4. DL8 and DL11 are in Zone 5 and DL12 is in Zone 6. If DL 1 is not safe lower dose levels will be considered, starting from DL B4.

Table 5

Gemcitabine	500mg/m2 (Day 1 and 8)	B13	B9	4	8	12
	350mg/m2 (Day 1 and 8)	B12	B8	3	7	11
	250mg/m2 (Day 1 and 8)	B11	B7	2	6	10
	175mg/m2 (Day 1 and 8)	B10	B6	**1**	5	9
	100mg/m2 (Day 1 and 8)	B5	B4	B1	B2	B3
Elimusertib (BAY 1895344)		10mg once daily (Days 2-3 and 9-10)	20mg once daily (Days 2-3 and 9-10)	20mg twice daily (Days 2-3 and 9-10)	30mg twice daily (Days 2-3 and 9-10)	40mg twice daily (Days 2-3 and 9-10)

Dose Level	Gemcitabine Dose	Cycle Length
-1	100mg/m2 (Day 1 and 8)	21 days
1	175mg/m2 (Day 1 and 8)	21 days
2	250mg/m2 (Day 1 and 8)	21 days
3	350mg/m2 (Day 1 and 8)	21 days
4	500mg/m2 (Day 1 and 8)	21 days

Dose Level	Elimusertib (BAY1895344)	Cycle Length
-2	10mg once daily (Days 2-3 and Days 9-10)	21 days
-1	20mg once daily (Days 2-3 and Days 9-10)	21 days
1	20mg twice daily (Days 2-3 and Days 9-10)	21 days
2	30mg twice daily (Days 2-3 and Days 9-10)	21 days
3	40mg twice daily (Days 2-3 and Days 9-10)	21 days

Operating rules are summarized below:

- If DL1 is safe open DL2 and DL5 in Zone 2

- In Zone 2: If only DL2 is safe open DL3 in Zone 3
- In Zone 2: If only DL5 is safe open DL9 in Zone 3
- In Zone 2: If both DL2 and DL5 are safe DL3, DL6, and DL9 could be opened in Zone 3

- In Zone 3: If only DL3 is safe open DL4 in Zone 4
- In Zone 3: if only DL3 and DL6 are safe open DL4 and DL7 in Zone4
- In Zone 3: If DL3, DL6 and DL9 are safe DL4, DL7, and DL10 could be opened
- In Zone 3: if only DL9 is safe, there is no further escalation
- In Zone 3: if only DL6 is safe, there is no further escalation

- In Zone 4: if both DL4 and DL7 are safe open DL8 in Zone 5
- In Zone 4: if both DL7 and DL10 are safe open DL11 in Zone 5
- In Zone 4: if DL4, DL7, and DL10 are safe open DL8 and DL 11 in Zone 5
- In Zone 4: if only DL4 is safe, no further escalation
- In Zone 4: if only DL7 is safe, no further escalation
- In Zone 4: if only DL10 is safe, no further escalation

- In Zone 5: if only DL8 is safe no further escalation
- In Zone 5: if only DL11 is safe no further escalation
- In Zone 5: if D8 and DL11 are safe open DL 12 in Zone 6

• If DL1 is not safe de-escalate to DL B4

• If DL B4 is safe open DL B6 and DL B1
• If both DL B6, and DL B1 are safe open DL B7 and DL B2

• If only DL B6 is safe open DL B7
• If only DL B1 is safe open DL B2

• If both DL B2 and DL B7 are safe open DL B3 and DL B8
• If only DL B2 is safe open DL B3
• If only DL B7 is safe open DL B8

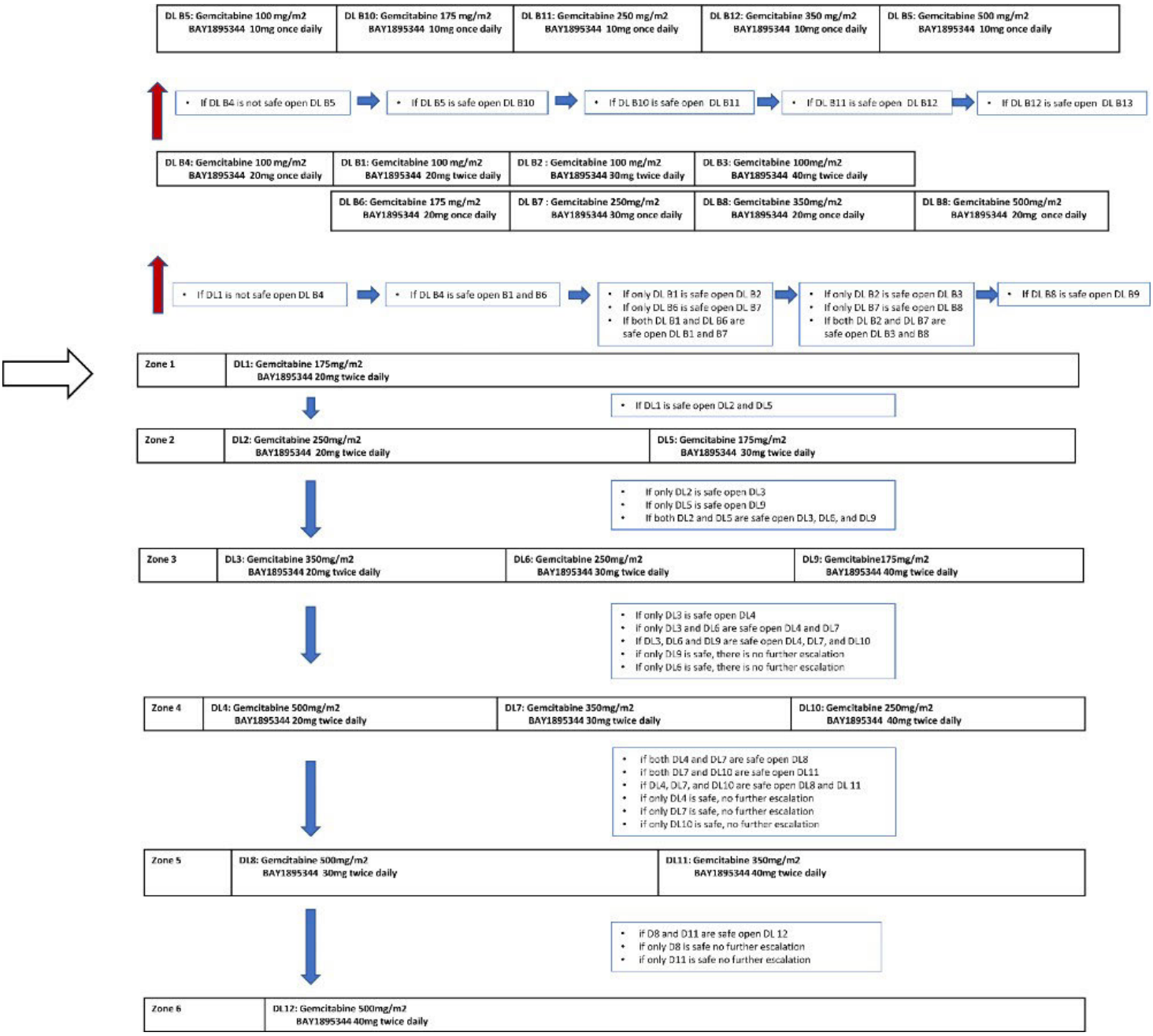
• If DL B8 is safe open DL B9

• If DL B4 is not safe de-escalate to B5

• If DL B5 is safe open DL B10
• If DL B10 is safe open DL B11
• If DL B11 is safe open DL B12
• If DL B12 is safe open DL B13

• If DL B5 is not safe stop the trial

Figure 5: Definition of the Zones and operating rules of the trial.



Example Dose Escalations

Example #1

Gemcitabine	500mg/m2 (Day 1 and 8)	B13	B9	4	8	12
	350mg/m2 (Day 1 and 8)	B12	B8	3	7	11
	250mg/m2 (Day 1 and 8)	B11	B7	2	6	10
	175mg/m2 (Day 1 and 8)	B10	B6	**1**	5	9
	100mg/m2 (Day 1 and 8)	B5	B4	B1	B2	B3
Elimusertib (BAY 1895344)		10mg once daily (Days 2-3 and 9-10)	20mg once daily (Days 2-3 and 9-10)	20mg twice daily (Days 2-3 and 9-10)	30mg twice daily (Days 2-3 and 9-10)	40mg twice daily (Days 2-3 and 9-10)

Three patients are enrolled in dose level 1 and there are no DLTs. Dose escalation then moves to dose level 2 and 5. Three patients are enrolled on each of these dose levels and again there are no DLTs. Dose levels 3, 6, and 9 could be opened. Since no more than two dose levels can be opened at one time, the two dose levels would be selected at the investigator's meetings.

Example #2

Gemcitabine	500mg/m2 (Day 1 and 8)	B13	B9	4	8	12
	350mg/m2 (Day 1 and 8)	B12	B8	3	7	11
	250mg/m2 (Day 1 and 8)	B11	B7	2	6	10
	175mg/m2 (Day 1 and 8)	B10	B6	**1**	5	9
	100mg/m2 (Day 1 and 8)	B5	B4	B1	B2	B3
Elimusertib (BAY 1895344)		10mg once daily (Days 2-3 and 9-10)	20mg once daily (Days 2-3 and 9-10)	20mg twice daily (Days 2-3 and 9-10)	30mg twice daily (Days 2-3 and 9-10)	40mg twice daily (Days 2-3 and 9-10)

Three patients are enrolled in dose level 1 and there are 2 DLTs. Dose escalation then moves to dose level B4. Three patients are enrolled on dose level B4 and again there are 2 DLTs. Dose levels B5 is then opened. None of the three patients enrolled at dose level B5 have a DLT. Dose escalation would then proceed to opening dose level B10.

Investigator Meetings

During dose escalation, a monthly teleconference will be held with the principal investigator, site study teams, study statistician and CTEP medical officer (or designee). At this meeting, the safety of the patients on the current cohort(s) will be reviewed and decisions about opening of new dose levels will be discussed. Following this meeting, the principal investigator will notify, in writing, CTEP the plan for opening of future dose levels.

Patient Assignment into Dose Levels

When new dose levels are opened, the following system be will be utilized to assign patients into dose level cohorts:

- If only one dose level is open, three patients will be enrolled in that dose level
- If more than two dose levels are open, three patients will be assigned to each of the two of the open dose

levels by randomization. These dose levels are decided during the Investigator Meetings.

Randomization

When two dose levels are open, patients will be randomly assigned to the dose levels (see Appendix 2 for details of the randomization procedure).

6.4 Dose Expansion Cohorts:

Once the RP2D is reached, the protocol will pause for an amendment, and then an additional 14 patients with pancreatic cancer and 14 patients with ovarian cancer will be treated at this dose. For the pancreatic cancer cohorts, at least 4 patients need to have ATM deficiency (defined as a deleterious germline genomic alteration of ATM or evidence of biallelic inactivation of ATM). For the expansion cohort, patients will continue to be monitored for occurrence of DLT. The DLT assessment window will again be during the first cycle (21 days). If 2 of the first 5 ovarian or pancreatic patients or if ≥2 of 6 ovarian or pancreatic 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 RP2D. 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.

6.5 General Concomitant Medication and Supportive Care Guidelines

Because elimusertib (BAY 1895344)is primarily metabolized by CYP3A4, drugs that are strong inhibitors or inducers of CYP3A4 should be avoided. Elimusertib (BAY 1895344) has the potential to inhibit and induce CYP3A4 and 2C19, inhibit CYP2C8, 2C9, P-gp, BCRP, OAT1B1, and OAT1B3. CYP3A4
substrates with narrow therapeutic window should be avoided, and substrates of CYP2C8, 2C9, 2C19, BCRP, P-gp, OATP1B1, and OATP1B3 should be used with caution.

Because there is a potential for interaction of elimusertib (BAY 1895344) 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.

Patients will be pre-treated with long-acting 5-HT3 antagonists (like palonosetron 0.25mg IV) before each gemcitabine infusion. They will also receive dexamethasone 4mg po QD on days they receive elimusertib (BAY 1895344). The dexamethasone will be administered approximately 0-60 minutes before the time of elimusertib (BAY 1895344) administration. Further treatment of nausea and diarrhea will be at the treating physician's discretion.

Because of pH dependent solubility, antacid drugs, H2-blockers, and proton pump inhibitors may affect absorption and exposure of elimusertib (BAY 1895344).

Treatment of anemia with transfusion of packed red blood cells is allowed.

Growth factor support with GCSF is allowed after the DLT assessment window (cycle 1). Growth factor support should be done according to institutional policy and the package label.

6.6 Duration of Therapy

In the absence of treatment delays due to adverse event(s), treatment may continue for indefinite duration 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 childbearing 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).

6.7 Duration of Follow-Up

Patients will be followed for 30 days after removal from study or until death, whichever occurs first. 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 ELIMUSERTIB (BAY 1895344) Dose Modification Levels

Starting Dose Level	Dose Reduction 1	Dose Reduction 2
10mg once daily (Days 2-3 and Days 9-10)	10mg once daily (Day 2 and Day 9)	No further reduction
20mg once daily (Days 2-3 and Days 9-10)	10mg once daily (Days 2-3 and Days 9-10)	10mg once daily (Day 2 and Day 9)
20mg twice daily (Days 2-3 and Days 9-10)	20mg once daily (Days 2-3 and Days 9-10)	10mg once daily (Days 2-3 and Days 9-10)
30mg twice daily (Days 2-3 and Days 9-10)	20mg twice daily (Days 2-3 and Days 9-10)	20mg once daily (Days 2-3 and Days 9-10)
40mg twice daily (Days 2-3 and Days 9-10)	30mg twice daily (Days 2-3 and Days 9-10)	20mg twice daily (Days 2-3 and Days 9-10)

Gemcitabine Dose Modification Levels

Starting Dose Level	Dose Reduction 1	Dose Reduction 2
100 mg/m^2	No further reduction	No further reduction
175 mg/m^2	100 mg/m^2	No further reduction
250 mg/m^2	175 mg/m^2	100 mg/m^2
350 mg/m^2	250 mg/m^2	175 mg/m^2
500 mg/m^2	350 mg/m^2	250 mg/m^2

Nausea	**Management/Next Dose for Elimusertib (BAY1895344)**	**Management/Next Dose for Gemcitabine**
≤ Grade 1	No change in dose	No change in dose
Grade 2	Hold until ≤ Grade 1. Resume at same dose level.*** Consider adding a Substance P-Neurokinin 1 (NK1) Receptor Antagonist (for example aprepitant IV or po starting on the day of gemcitabine infusion)	Hold until ≤ Grade 1. Resume at same dose level.*** Consider adding a Substance P-Neurokinin 1 (NK1) Receptor Antagonist (for example aprepitant IV or po starting on the day of gemcitabine infusion)
Grade 3	Hold* until < Grade 2. Resume at one dose level lower (if maximal anti-nausea medications were previously used).** Consider adding a Substance P-Neurokinin 1 (NK1) Receptor Antagonist (for example aprepitant IV or po starting on the day of gemcitabine infusion)	Hold* until < Grade 2. Resume at one dose level lower (if maximal anti-nausea medications were previously used) Consider adding a Substance P-Neurokinin 1 (NK1) Receptor Antagonist (for example aprepitant IV or po starting on the day of gemcitabine infusion)
Grade 4	Off protocol therapy	Off protocol therapy
*Patients requiring a delay of >2 weeks should go off protocol therapy. **Patients requiring > two dose reductions of **elimusertib (BAY1895344)** should go off protocol therapy.		

Recommended management: antiemetics.
***The recommendations are not mandatory and clinicians can use their judgement.

When the treating clinician believes that both gemcitabine and elimusertib (BAY1895344) have contributed to the toxicity, then both drugs should be modified according to the table above

At the discretion of the treating investigator, after cycle 1, elimusertib (BAY1895344) can be administered without gemcitabine.

Vomiting	**Management/Next Dose for elimusertib (BAY1895344)**	**Management/Next Dose for Gemcitabine**
≤ Grade 1	No change in dose	No change in dose
Grade 2	Hold until ≤ Grade 1. Resume at same dose level.*** Consider adding a Substance P-Neurokinin 1 (NK1) Receptor Antagonist (for example aprepitant IV or po starting on the day of gemcitabine infusion)	Hold until ≤ Grade 1. Resume at same dose level.*** Consider adding a Substance P-Neurokinin 1 (NK1) Receptor Antagonist (for example aprepitant IV or po starting on the day of gemcitabine infusion)
Grade 3	Hold* until < Grade 2. Resume at one dose level lower (if maximal anti-nausea medications were previously used).** Consider adding a Substance P-Neurokinin 1 (NK1) Receptor Antagonist (for example aprepitant IV or po starting on the day of gemcitabine infusion)	Hold* until < Grade 2. Resume at one dose level lower (if maximal anti-nausea medications were previously used). Consider adding a Substance P-Neurokinin 1 (NK1) Receptor Antagonist (for example aprepitant IV or po starting on the day of gemcitabine infusion)
Grade 4	Off protocol therapy	Off protocol therapy

*Patients requiring a delay of >2 weeks should go off protocol therapy.
Patients requiring > two dose reductions of **elimusertib (BAY1895344) should go off protocol therapy.
*** These grade 2 recommendations are not mandatory, and clinicians can use their judgement

When the treating clinician believes that both gemcitabine and elimusertib (BAY1895344) have contributed to the toxicity, then both drugs should be modified according to the table above.

At the discretion of the treating investigator, after cycle 1, elimusertib (BAY1895344) can be administered without gemcitabine.

Diarrhea	**Management/Next Dose for elimusertib (BAY1895344)**	**Management/Next Dose for Gemcitabine**
≤ Grade 1	No change in dose	No change in dose
Grade 2	Hold until ≤ Grade 1. Resume at same dose level.***	Hold until ≤ Grade 1. Resume at same dose level.***
Grade 3	Hold* until < Grade 2. If maximal anti-diarrheal medications were used, resume at one dose level lower**	Hold* until < Grade 2. If maximal anti-diarrheal medications were used, resume at one dose level lower**
Grade 4	Off protocol therapy	Off protocol therapy
*Patients requiring a delay of >2 weeks should go off protocol therapy. **Patients requiring > two dose reductions of **elimusertib (BAY1895344)** should go off protocol therapy.		
Recommended management: Loperamide antidiarrheal therapy Dosage schedule: 4 mg at first onset, followed by 2 mg with each loose motion until diarrhea-free for 12 hours (maximum dosage: 16 mg/24 hours) Adjunct anti-diarrheal therapy is permitted and should be recorded when used. *** These grade 2 recommendations are not mandatory, and clinicians can use their judgement. When the treating clinician believes that both gemcitabine and elimusertib (BAY1895344) have contributed to the toxicity, then both drugs should be modified according to the table above At the discretion of the treating investigator, after cycle 1, elimusertib (BAY1895344) can be administered without gemcitabine.		

Non-Hematological Toxicities	**Management/Next Dose for Elimusertib (BAY1895344)**	**Management/Next Dose for Gemcitabine**
≤ Grade 1	No change in dose	No change in dose
Grade 2	At clinician's discretion, patient can be treated at the same dose level***	At clinician's discretion, patient can be treated at the same dose level***
Grade 3*****	Hold* until < Grade 2. Resume at one dose level lower.**	Hold* until < Grade 2. Resume atone dose level lower.
Grade 4*****	Hold* until < Grade 2. Case must be discussed with principal investigator to ensure that it is safe to resume dosing at one dose level lower.**	Hold* until < Grade 2. Case must be discussed with principal investigator to ensure that it is safe to resume dosing at one dose level lower.**

Patients requiring a delay of >2 weeks should go off protocol therapy.
Patients requiring > two dose reductions of **elimusertib (BAY1895344) should go off protocol therapy.
Recommended management: antiemetics.
*** These grade 2 recommendations are not mandatory, and clinicians can use their judgement.
****When the treating clinician believes that both gemcitabine and elimusertib (BAY1895344) have contributed to the toxicity, then both drugs should be modified according to the table above
***** This excludes alopecia and other toxicities that are not clinically significant. Grade 3 hyponatremia, hypokalemia, hypomagnesemia and hypophosphatemia require dose reductions only if they continue for ≥ 5 days despite maximal medical management.

At the discretion of the treating investigator, after cycle 1, elimusertib (BAY1895344) can be administered without gemcitabine.

Neutropenia on Day of Study Drug Dosing	**Management/Next Dose for elimusertib (BAY1895344)**	**Management/Next Dose for Gemcitabine**
≤ Grade 1	No change in dose	No change in dose
Grade 2	Hold until ≤ Grade 1. Resume atsame dose level.***	Hold until ≤ Grade 1. Resume at same dose level.***
Grade 3	Hold* until < Grade 2. Resume at one dose level lower, if indicated.**	Hold* until < Grade 2. Resume at one dose level lower, if indicated.
Grade 4	Hold* until < Grade 2. Resume at one dose level lower, if indicated.**	Hold* until < Grade 2. Resume at one dose level lower, if indicated.

*Patients requiring a delay of >2 weeks should go off protocol therapy.
Patients requiring > two dose reductions of **elimusertib (BAY1895344) should go off protocol therapy.
*** These grade 2 recommendations are not mandatory, and clinicians can use their judgement.

When the treating clinician believes that both gemcitabine and elimusertib (BAY1895344) have contributed to the toxicity, then both drugs should be modified according to the table above

At the discretion of the treating investigator, after cycle 1, elimusertib (BAY1895344) can be administered without gemcitabine.

Thrombocytopenia on Day of Study Drug Dosing	**Management/Next Dose for elimusertib (BAY1895344)**	**Management/Next Dose for Gemcitabine**
≤ Grade 1	No change in dose	No change in dose
Grade 2	Hold until ≤ Grade 1. Resume at same dose level.	Hold until ≤ Grade 1. Resume at same dose level.
Grade 3	Hold* until < Grade 2. Resume at one dose level lower, if indicated.**	Hold* until < Grade 2. Resume at one dose level lower, if indicated.
Grade 4	Hold* until < Grade 2. Resume at one dose level lower, if indicated.**	Hold* until < Grade 2. Resume at one dose level lower, if indicated.

*Patients requiring a delay of >2 weeks should go off protocol therapy.
Patients requiring > two dose reductions of **elimusertib (BAY1895344) should go off protocol therapy.

When the treating clinician believes that both gemcitabine and elimusertib (BAY1895344) have contributed to the toxicity, then both drugs should be modified according to the table above

At the discretion of the treating investigator, after cycle 1, elimusertib (BAY1895344) can be administered without gemcitabine.

Gemcitabine should be permanently discontinued in patients who experience or develop the following:

- Unexplained clinically significant dyspnea, with or without bronchospasm, or severe pulmonary toxicity
- Hemolytic uremic syndrome (HUS) or severe renal impairment
- Severe hepatic toxicity
- Capillary leak syndrome (CLS)
- Posterior reversible encephalopathy (PRES)
- Thrombotic Thrombocytopenic Purpura (TTP)

8. PHARMACEUTICAL INFORMATION

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

8.1 CTEP IND Agent

8.1.1 Elimusertib (BAY 1895344) (NSC 810486)

Chemical Name: 2-[(3R)-3-methylmorpholin-4-yl]-4-(1-methyl-1H-pyrazol-5-yl)-8-(1H-pyrazol5-yl)-1,7-naphthyridine

Classification*:* ATR inhibitor

CAS Registry Number: 1876467-74-1

Molecular Formula: $C_{20}H_{21}N_7O$ **M.W.:** 375 g/mol

Approximate Solubility: Elimusertib (BAY1895344) is practically insoluble in water at pH≥4.5, slightly soluble in ethanol, acetonitrile and acetone and sparingly soluble in 0.1 N HCl and polyethylene glycol (PEG)400.

Mode of Action*:* Elimusertib (BAY1895344) is a potent, selective inhibitor of ataxia telangiectasia and Rad3-related (ATR) pathway. Activation of the ATR pathway may be critical for survival in tumor harboring defects in DNA repair or DNA damage signaling pathways.

Description: Yellow solid.

How Supplied: Bayer supplies and the Pharmaceutical Management Branch, CTEP, DCTD, NCI distributes elimusertib (BAY 1895344) as oral film-coated tablets. Tablets will be packaged in bottles containing 18 tablets.

Strength	Description	Availability
20 mg	film-coated tablets are red and round with 6 mm diameter	Not available after July 31, 2024
10 mg	film-coated tablets are red and round with 5 mm diameter	Not available after February 28, 2025

Tablet core components include active drug substance, microcrystalline cellulose, lactose monohydrate, croscarmellose sodium, colloidal silicon dioxide, and magnesium stearate. Film

coating contains hydroxypropyl methylcellulose (hypromellose), macrogol, titanium dioxide, and ferric oxide red.

Storage: Store at or below 25 °C. Do not freeze.

If a storage temperature excursion is identified, promptly return elimusertib (BAY 1895344) to 25 °C or below 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 are on-going. Dispense in original package or repackaging is only allowed using 45 mL plastic HDPE white bottle sealed with PP/PP white child-resistant screw cap.

Route of Administration: **Oral**

Method of Administration: Swallow tablets immediately following removal from package. Take elimusertib (BAY 1895344)one (1) hour before or two (2) hours after a meal with approximately 240 mL of water.

Potential Drug Interactions:
No formal DDI studies have been conducted with elimusertib (BAY 1895344) in humans.

In vitro, elimusertib (BAY 1895344) is metabolized mainly by CYP3A4 and to a much lesser extent CYP1A1. Elimusertib (BAY 1895344) is not a P-gp or BCRP substrate. Strong inhibitors and inducers of CYP3A4 should be avoided.

Elimusertib (BAY 1895344) is a weak to moderate inhibitor of CYP3A4, 2C8, 2C9, and 2C19 *in vitro*. Elimusertib (BAY 1895344) had no significant inhibitory effect on CYP1A2, 2A6, 2B6, 2D6, and 2E1. *In vitro*, elimusertib (BAY 1895344) was also shown to be an inducer of CYP3A4 and 2C19 but not 1A2. Elimusertib (BAY 1895344) is predicted to be a moderate inducer toward sensitive CYP3A4 substrates. CYP3A4 substrates with narrow therapeutic index should be avoided, and substrates of CYP2C8, 2C9, and 2C19 should be used with caution.

Elimusertib (BAY 1895344) was identified as an *in vitro* inhibitor of BCRP, P-gp, BSEP, OATP1B1, and OATP1B3. The risk for clinically relevant drug-drug interactions due to inhibition of BSEP is considered to be negligible but substrates of BCRP, P-gp, OATP1B1, and OATP1B3 should beused with caution.

Because of pH dependent solubility, antacid drugs, H2-blockers, and proton pump inhibitors may affect absorption and exposure ofelimusertib (BAY 1895344).

Patient Care Implications:

- Women of childbearing potential and their male partners should use appropriate contraceptive measures during and for 6 months after last study drug administration.
- Breast-feeding should be discontinued during treatment and for 4 months after last study drug administration.
- Based on the UV absorption properties of elimusertib (BAY1895344), patients should be instructed to use topical sun block and UV-blocking sunglasses during treatment with elimusertib (BAY1895344). Direct exposure of patients to sun light after administration

should be avoided.

Availability

Elimusertib (BAY1895344) is an investigational agent supplied to investigators by the Division of Cancer Treatment and Diagnosis (DCTD), NCI.

Elimusertib (BAY1895344)is provided to the NCI under a Collaborative Agreement between the Pharmaceutical Collaborator and the DCTD, NCI (see Section 13.5).

8.1.2 Agent Ordering and Agent Accountability

8.1.2.1 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.

Sites can place orders for PMB-supplied agents only after enrollment onto the study. Please provide the patient ID# when placing an order.

Submit agent requests through the PMBAURORA application. Access to AURORA 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.

8.1.2.2 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.

8.1.3 Investigator Brochure Availability

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

8.1.4 Useful Links and Contacts

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

8.2 Commercial Agent

8.2.1 Gemcitabine (NSC 613327)

8.2.1.1 Product description

Gemcitabine (gemcitabine for injection, USP) is a nucleoside metabolic inhibitor that exhibits antitumor activity. Gemcitabine HCl is 2′-deoxy-2′,2′-difluorocytidine monohydrochloride (β-isomer). The empirical formula for gemcitabine HCl is C9H11F2N3O4 • HCl. It has a molecular weight of 299.66 (Gemcitabine Package Insert, 2019).

Gemcitabine HCl is soluble in water, slightly soluble in methanol, and practically insoluble in ethanol and polar organic solvents.

Gemcitabine is supplied in a sterile form for intravenous use only

Lyophilized form: Vials of gemcitabine contain either 200 mg or 1 g of gemcitabine HCl (expressed as free base) formulated with mannitol (200 mg or 1 g, respectively) and sodium acetate (12.5 mg or 62.5 mg, respectively) as a sterile lyophilized powder. Hydrochloric acid and/or sodium hydroxide may have been added for pH adjustment.

Unopened vials of gemcitabine are stable until the expiration date indicated on the package when stored at controlled room temperature 20° to 25°C (68° to 77°F) and that allows for excursions between 15° and 30°C (59° and 86°F).

Exercise caution and wear gloves when preparing gemcitabine solutions. Immediately wash the skin thoroughly or rinse the mucosa with copious amounts of water if gemcitabine contacts the skin or mucus membranes. Death has occurred in animal studies due to dermal absorption.

Solution for Injection: Vials of Gemcitabine Injection contain either 200 mg, 1 g, or 2 g of gemcitabine HCl (expressed as free base). Each mL contains equivalent of 38 mg of gemcitabine in Water for Injection, USP. Hydrochloric acid and/or sodium hydroxide may have been added for pH adjustment.

Unopened vials of Gemcitabine Injection are stable until the expiration date indicated on the

package when stored at 2° to 8°C (36° to 46°F). Do not freeze.

Exercise caution and wear gloves when preparing gemcitabine solutions. Immediately wash the skin thoroughly with soap and water or rinse the mucosa with copious amounts of water if gemcitabine contacts the skin or mucus membranes. Death has occurred in animal studies due to dermal absorption.

8.2.1.2 Solution Preparation

Lyophilized form: Reconstitute the vials with 0.9% Sodium Chloride Injection without preservatives.

Add 5 mL to the 200-mg vial or 25 mL to the 1-g vial. These dilutions each yield a Gemcitabine concentration of 38 mg/mL. Complete withdrawal of the vial contents will provide 200 mg or 1 g of gemcitabine. Prior to administration the appropriate amount of drug must be diluted with 0.9% Sodium Chloride Injection. Final concentrations may be as low as 0.1 mg/mL.

Reconstituted gemcitabine is a clear, colorless to light straw-colored solution. Inspect visually prior to administration and discard for particulate matter or discoloration. Gemcitabine solutions are stable for 24 hours at controlled room temperature of 20° to 25°C (68° to 77°F). Do not refrigerate as crystallization can occur. No incompatibilities have been observed with infusion bottles or polyvinyl chloride bags and administration sets.

Solution for Injection: Gemcitabine Injection is a clear and colorless to light straw-colored solution available in sterile single-use vials containing: 200 mg/5.26 mL, 1 g/26.3 mL, or 2 g/52.6 mL.

Each vial contains a gemcitabine concentration of 38 mg/mL. Hence, withdrawing 5.26 mL, 26.3 mL, or 52.6 mL of the vial contents will provide 200 mg, 1 g, or 2 g of gemcitabine, respectively. The appropriate amount of drug should be further diluted with 0.9% Sodium Chloride Injection to concentrations as low as 0.1 mg/mL.

After dilution with 0.9% Sodium Chloride Injection the solution should be inspected visually for particulate matter and discoloration, prior to administration, whenever solution or container permits. If particulate matter or discoloration is found, do not administer.

8.2.1.3 Route of administration:

Gemcitabine is supplied in a sterile form for intravenous use only.

Instructions for the administration of gemcitabine:

- Gemcitabine will be administered by IV infusion over 30 minutes (study sites may follow standard of care dosing windows)
- Gemcitabine will be administered on days 1 and 8 of each 21 day cycle
- Body surface area to be calculated per institutional standards of practice
- The gemcitabine dose will calculated per institutional standards of practice. In

calculating the dose, there will be no downward adjustment to "ideal" body weight unless institutional policy requires it.

8.2.1.4 Agent Ordering:

Commercially available from various manufacturers. See package insert for further information.

9. STATISTICAL CONSIDERATIONS

9.1 Study Design/Endpoints

The toxicity of the combination of gemcitabine plus elimusertib (BAY1895344)will be assessed with CTCAE
v. 5.0 criteria.

Each expansion cohort will enroll 14 participants. The two expansion cohorts will be used as a preliminary gauge of efficacy and will test the overall response rate (ORR), DoR, overall survival (OS) and PFS rates for the combination among the two groups. Radiological response will be assessed with RECIST 1.1 criteria and will be graded as complete response (CR), partial response (PR), stable disease (SD) and progressive disease (PD). DoR will be measured from the time at which measurement criteria are met for CR or PR (whichever is first recorded) until the first date on which recurrent or progressive disease is objectively documented. PFS will be defined as the time from study enrollment until the identification of disease progression or death, and OS will be defined as the time from study enrollment until death due to any cause. Subjects without disease progression or death at the time of analysis will be censored at the date of the last disease evaluation/date last known alive.

9.2 Sample Size/Accrual Rate

The estimated sample size for dose escalation is up to 36 patients. Subsequently the estimated subject accrual for dose expansion is 14 pancreatic cancer patients and 14 ovarian cancer patients. The total sample size for both phases of the trial is up to 64 patients, with an estimated accrual of 2-3 patients per month.

DOMESTIC PLANNED ENROLLMENT REPORT (TREATMENT)					
Racial Categories	Ethnic Categories				Total
	Not Hispanic or Latino		Hispanic or Latino		
	Female	Male	Female	Male	
American Indian/ Alaska Native	0	0	0	0	0
Asian	1	1	1	1	4
Native Hawaiian or Other Pacific Islander	0	0	0	0	0
Black or African American	1	1	1	1	4
White	27	25	2	2	56
More Than One Race	0	0	0	0	0
Total	29	27	4	4	64

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9.3 Stratification Factors

N/A

9.4 Analysis of Secondary Endpoints

A PK profile of elimusertib (BAY1895344) in combination with gemcitabine will be obtained on of this study for the escalation phase. Individual PK parameters will be estimated for the maximum concentration (C_{max}), area under the concentration-time curve (AUC), half-life ($t_{1/2}$), apparent clearance (Cl/F) and apparent volume of distribution (V/F), as feasible with non-compartmental

methods. The PK variables will be tabulated, and descriptive statistics (e.g., geometric means and coefficients of variation) will be calculated for each dose level. PK parameters will be reported descriptively.

Gemcitabine PK will be determined on day 1 and day 8. The CDA phenotype will be correlated with gemcitabine half-life, AUC and the dFdU/gemcitabine metabolic ratio

Paired pre-treatment and on-treatment tumor biopsies, which are mandatory in the expansion cohort, will allow us to test whether the gemcitabine/elimusertib (BAY1895344) increases DNA damage. We will assess this secondary endpoint of our expansion cohorts by assessing whether immunohistochemical markers of DNA damage, ɣH2AX and pNBS1, increase in on-treatment biopsies compared to the levels seen in pre-treatment biopsies. Because of the challenges of obtaining adequate tumor sample on both the pre-treatment and on-treatment biopsy, the expansion cohort will have 14 patients to ensure that there are an adequate number of paired biopsy samples to analyze.

Biopsies will be characterized for (1) the presence or absence of HR repair proficiency, defined according to formation of RAD51 foci; (2) presence or absence of replication stress, defined at the gene expression and protein levels for pKAP1, pRPA32, cyclin E and MYC; (3) presence or absence of ATR activation, defined by the expression of pATR and pCHK1; (4) DNA fiber assays will be used to assess whether gemcitabine/elimusertib (BAY1895344) treatment converts a cancer with stable replication forks to one with unstable replication forks. These characteristics will be evaluated in relation to the clinical outcomes of ORR and PFS time. We will use logistic regression to investigate associations with the binary outcome of response rate and Cox proportional hazard regression for the time to event analyses of PFS, with adjustment for relevant covariates including age, sex, race/ethnicity, ECOG performance status, metastatic sites and, in the niraparib trial, prior lines of therapy. Analyses of biopsies at the time of tumor resistance will be descriptive in nature, and secondary genomic alterations (e.g., reversion mutations) or gene expression changes relative to pre-treatment samples will be noted.

Exploratory correlative studies with pharmacodynamic (biological endpoints, toxicity and efficacy) will be analyzed with nonparametric statistics. Advanced population PK methods may be used at a later stage to assess the link between drug exposure and biological effects and efficacy.

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 CAEPRs for CTEP IND Agent

10.1.1.1 CAEPR for elimusertib (BAY 1895344)(NSC 810486)

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 197 patients.* Below is the CAEPR for elimusertib (BAY 1895344)**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 2.1, September 5, 2020[1]

Adverse Events with Possible Relationship to elimusertib (BAY 1895344)(CTCAE 5.0 Term) [n= 197]			**Specific Protocol Exceptions to Expedited Reporting (SPEER)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
Anemia			***Anemia (Gr 2)***
GASTROINTESTINAL DISORDERS			
	Diarrhea		
Nausea			***Nausea (Gr 2)***
	Vomiting		***Vomiting (Gr 2)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
Fatigue			***Fatigue (Gr 2)***
INVESTIGATIONS			
Neutrophil count decreased			***Neutrophil count decreased (Gr 2)***
Platelet count decreased			***Platelet count decreased (Gr 2)***
White blood cell decreased			***White blood cell decreased (Gr 2)***
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		

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

Adverse events reported on elimusertib (BAY 1895344)trials, but for which there is insufficient evidence to suggest that there was a reasonable possibility that elimusertib (BAY 1895344)caused the adverse event:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Febrile neutropenia
GASTROINTESTINAL DISORDERS - Abdominal pain; Dysphagia
INFECTIONS AND INFESTATIONS - Shingles
INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Injury, poisoning and procedural complications - Other (medication error)
INVESTIGATIONS - Alanine aminotransferase increased; Aspartate aminotransferase increased; Lipase increased; Lymphocyte count decreased; Serum amylase increased
METABOLISM AND NUTRITION DISORDERS - Hypokalemia; Hypophosphatemia
NERVOUS SYSTEM DISORDERS - Dysgeusia; Presyncope
PSYCHIATRIC DISORDERS - Irritability
RENAL AND URINARY DISORDERS - Proteinuria
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Epistaxis
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Rash maculo-papular
VASCULAR DISORDERS - Hypotension

Note: elimusertib (BAY 1895344) in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

10.1.2 Adverse Event List for Commercial Agent

10.1.2.1 Gemcitabine

The most common adverse reactions for gemcitabine (≥20%) are nausea/vomiting, anemia, increased aspartate aminotransferase (AST), increased alanine aminotransferase (ALT), neutropenia, increased alkaline phosphatase, proteinuria, fever, hematuria, rash, thrombocytopenia, dyspnea, and edema. Please refer to the gemcitabine package insert for additional information.

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.
  - Other AEs for the protocol that do not require expedited reporting are outlined in Section 10.3.4.

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

10.3 Expedited Adverse Event Reporting

10.3.1 Rave-CTEP-AERS Integration

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

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

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

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

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

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

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

using the direct link from Medidata Rave.

- Additional information about the CTEP-AERS integration is available on the CTSU website:Study specific documents: *Protocols > Documents> Protocol Related Documents> Adverse Event Reporting*, and
- Additional resources: *Resources > CTSU Operations Information> User Guides & Help Topics*.

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

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

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 <u>both</u> routine and expedited reporting, regardless of causality as long as the death occurred within 30 days after the last administration of theinvestigational 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 Administrationof the Investigational Agent/Intervention [1, 2]

FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)

NOTE: Investigators **MUST** immediately report to the sponsor (NCI) **ANY** SAEs, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64).

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

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

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

Grade 1-2 Timeframes	Grade 3-5 Timeframes
24-Hour notification, 10 Calendar Days	24-Hour notification, 5 Calendar Days

NOTE: Protocol-specific exceptions to expedited reporting of SAEs are found in the Specific Protocol Exceptions to Expedited Reporting (SPEER) portion of the CAEPR.

Expedited AE reporting timeframes are defined as:

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

[1]SAEs that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:
Expedited 24-Hour notifications are required for all SAEs followed by a complete report

- Within 5 calendar days for Grade 3-5 SAEs
- Within 10 calendar days for Grade 1-2 SAEs

[2]For studies using nuclear medicine or molecular imaging IND agents (NM, SPECT, or PET), the SAE reporting period is limited to 10 radioactive half-lives, rounded UP to the nearest whole day, after the agent/intervention was last administered. Footnote "1" above applies after this reporting period.

Effective Date: August 30, 2024

10.3.4 Adverse Events of Special Interest

Adverse Event Special Interest (AESIs) are a subset of events of scientific and/or medical concern specific to this protocol, for which ongoing monitoring and communication by the Investigator to the Sponsor is required.

Adverse Events of Special Interest (AESIs) for this study are related to the biopsy component of the study. These events should be captured in Rave as they occur and should be reported expeditiously according to the table above. Four of these AESIs are not currently part of the CTCAE and must be reported in Rave/CTEP-AERS using the term: Injury, poisoning and

procedural complications - Other, specify. The AESI Terms and Grading Table below (Section 10.3.4.1) lists the AESI terms and their grading. The AESI Reporting Table (Section 10.3.4.2) provides instruction on how to record these events in Rave/CTEP-AERS.

10.3.4.1 Adverse Event Special Interest (AESI) Terms and Grading

Adverse Event Special Interest (AESI) Terms and Grading

AESI	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Definition
Biopsy-related hemorrhage	Symptomatic hematoma requiring medications	Any hematoma (symptomatic or asymptomatic) requiring unplanned observation with less than 24 hours of hospitalization	Hemorrhage requiring transfusion, radiologic, endoscopic or surgical intervention or greater than 24 hours of hospitalization	Hemorrhage with life-threatening consequences; urgent intervention indicated	Death	A disorder characterized by bleeding related to a biopsy procedure.
Biopsy-related nerve injury	Symptomatic nerve pain or injury requiring medications	Any nerve injury requiring unplanned observation with less than 24 hours of hospitalization	Any nerve injury requiring surgical intervention or greater than 24 hours of hospitalization	Nerve injury with life-threatening consequences; urgent intervention indicated	Death	A finding of damage to a nerve related to a biopsy procedure.
Biopsy-related organ injury	Symptomatic organ pain or injury requiring medications	Any organ injury requiring unplanned observation with less than 24 hours of hospitalization	Any organ injury that requires radiologic, endoscopic or surgical intervention or greater than 24 hours of hospitalization	Organ injury with life-threatening consequences; urgent intervention indicated	Death	A finding of damage to an organ related to a biopsy procedure.
Biopsy-related anesthesia (local or moderate sedation) effects	Symptomatic anesthesia effects requiring medications	Any biopsy-related anesthesia effects requiring medical intervention for reversal of symptomatic effects and/or requiring unplanned observation with less than 24 hours of hospitalization	Any anesthesia effects requiring intervention for reversal or treatment of symptomatic effects and/or requiring greater than 24 hours of hospitalization	Anesthesia effects with life-threatening consequences; urgent intervention indicated	Death	A disorder characterized by reactions related to the administration of local or moderate sedation given for a biopsy procedure.
Pneumothorax	Asymptomatic; clinical or diagnostic observations only; intervention not indicated	Symptomatic; intervention indicated	Sclerosis and/or operative intervention indicated; hospitalization indicated	Life-threatening consequences; urgent intervention indicated	Death	A disorder characterized by abnormal presence of air in the pleural cavity resulting in the collapse of the lung.
Pain	Mild pain	Moderate pain; limiting instrumental ADL	Severe pain; limiting self care ADL	-	-	A disorder characterized by the sensation of marked discomfort, distress or agony.
Wound infection	Localized, local intervention indicated	Oral intervention indicated (e.g., antibiotic, antifungal, or antiviral)	IV antibiotic, antifungal, or antiviral intervention indicated; invasive intervention indicated	Life-threatening consequences; urgent intervention indicated	Death	A disorder characterized by an infectious process involving the wound.
Allergic reaction	Systemic intervention not indicated	Oral intervention indicated	Bronchospasm; hospitalization indicated for clinical sequelae; intravenous intervention indicated	Life-threatening consequences; urgent intervention indicated	Death	A disorder characterized by an adverse local or general response from exposure to an allergen.

10.3.4.2 Adverse Event Special Interest (AESI) Reporting

Adverse Event Special Interest (AESI) Reporting

AESI Term	CTCAE term to Select	Other, specify language to record:
Pneumothorax*	Pneumothorax	N/A
Pain*	Pain	N/A
Wound infection*	Wound infection	N/A
Allergic reaction*	Allergic reaction	N/A
Biopsy-related hemorrhage**	Injury, poisoning and procedural complications - Other, specify	Biopsy-related hemorrhage
Biopsy-related nerve injury**	Injury, poisoning and procedural complications - Other, specify	Biopsy-related nerve injury
Biopsy-related organ injury**	Injury, poisoning and procedural complications - Other, specify	Biopsy-related organ injury
Biopsy-related anesthesia (local or moderate sedation) effects**	Injury, poisoning and procedural complications - Other, specify	Biopsy-related anesthesia (local or moderate sedation) effects

*Select the indicated current CTCAE Term.

**Select CTCAE term, "Injury, poisoning and procedural complications - Other, specify" and fill in the 'Specify' with the AESI term when prompted in Rave.

For example, if the subject experienced a biopsy-related hemorrhage, select Injury, poisoning and procedural complications - Other, specify and write in 'Biopsy-related hemorrhage' when prompted in Rave and/or CTEP-AERS.

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 1 week prior to start of protocol therapy. Scans and x-rays must be done ≤4 weeks prior to the start of therapy. In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next cycle of therapy.

		Cycle 1							Cycle 2+							
	Pre-Study	Day 1	Day 2	Day 3	Day 8	Day 9-10	Day 15-17	Day 21	Day 1	Day 2	Day 3	Day 8	Day 9-10	Day 15-17	Day 21	Off Study[a]
Elimusertib (BAY1895344)		A*	A	A	A*	A			A*	A	A	A*	A			
Gemcitabine		B*			B*				B*			B*				
Informed consent	X															
Demographics	X															
Medical history	X															
Concurrent meds	X	X	----	----	----	----	----	----	----	----	----	----	----	----	X	
Physical exam	X	X							X							X
Vital signs	X	X							X							X
Height	X	X							X							
Weight	X	X							X							X
Performance status	X	X							X							X
CBC w/diff, plts[b]	X	X			X				X			X				X
Comprehensive chemistry panel [b,c]	X	X			X				X			X				X
EKG (as indicated)	X															
Adverse event evaluation	X	X	----	----	----	----	----	----	----	----	----	----	----	----	----	X
Tumor measurements	X	Tumors will be measured and confirmed ≤4 weeks before the start of treatment weeks. Documentation (radiologic) must be provided for patients removed from study for progressive disease.														
Radiologic evaluation	X	Radiologic measurements will be performed following cycle 2 (6 weeks +/- 1 week) and then should be performed every 9 weeks (+/- 1 week).														
Pregnancy test[d]	X															
Completion of Medication Diary		X	X	X	X	X			X	X	X	X	X			
Collection of Archival Tissue[k]	X															
PK blood collection		X[e/g]	X[l/m]		X[f/h]											

		Cycle 1							Cycle 2+							
	Pre-Study	Day 1	Day 2	Day 3	Day 8	Day 9-10	Day 15-17	Day 21	Day 1	Day 2	Day 3	Day 8	Day 9-10	Day 15-17	Day 21	Off Study[a]
Blood Collection Streck cfDNA tube	X					X[i,j]			X[i,j]							X[i,k]
Biopsy[k]	X					X										X[i]

A Elimusertib (BAY1895344): Dose as assigned; Days 2-3 and Days 9-10 of each 21 day cycle. There is a +/-1 window for administration of gemcitabine/elimusertib (BAY1895344). Elimusertib (BAY1895344) is administered on the two days following each gemcitabine infusion.

..B: Gemcitabine*:* Dose as assigned; D1 and D8 (of every 21-day cycle).

a: Off-study evaluation 30 days after completion of study treatment.

b: To be performed within a ±3 day window; for CBC, to be performed up to 3 days prior to scheduled day.

c: Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, LDH, phosphorus, potassium, total protein, SGOT [AST], SGPT [ALT], sodium.

d: Urine or Serum pregnancy test for women of childbearing potential.

e: Dose Escalation: C1D1 pre-dose, 25 min pre end of infusion, 1hr (+/- 10 min), 1.5hr (+/- 10 min)

f: Dose Escalation: C1D8 pre-dose, 25 min pre end of infusion

g: Dose Expansion: C1D1 pre-dose, 25 min pre end of infusion, 1hr (+/- 10 min), 1.5hr (+/- 10 min)

h: Dose Expansion: C1D8 pre-dose

i: Optional

j: Dose expansion only: C1D9, C3D1

k: Dose expansion only

l: Dose Escalation: C1D2 pre-dose, 25 minutes, 1 hr (+/- 10 min), 1.5 hr (+/- 10 min), 2hr (+/- 10 min), 4 hr (+/- 10 min), 6 hr (+/- 10 min)

m: Dose Expansion: C1D2 pre-dose, 25 minutes, 1 hr (+/- 10 min), 1.5 hr (+/- 10 min)

*: Gemcitabine and elimusertib (BAY1895344) can be administered of each cycle on Day 1 +/- 1 day. It can also be administered of each cycle on Day 8 +/- 1 day.

12. MEASUREMENT OF EFFECT

Although the clinical benefit of this drug(s) has not yet been established, the intent of offering this treatment is to provide a possible therapeutic benefit, and thus the patient will be carefully monitored for tumor response and symptom relief in addition to safety and tolerability. Patients with measurable disease will be assessed by standard criteria. For the purposes of this study, patients should be re-evaluated every 6 weeks for cycle 1-2 then every 9 weeks. In addition to a baseline scan, confirmatory scans will also be obtained 6 weeks for cycle 1-2 then every 9 weeks following initial documentation of an objective response, and then every third cycle after.

12.1 Antitumor Effect – Solid Tumors

For the purposes of this study, patients should be re-evaluated for response every 9 weeks. In addition to a baseline scan, confirmatory scans should also be obtained 9 weeks following initial documentation of an objective response.

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

12.2 Definitions

Evaluable for Toxicity. All patients will be evaluable for toxicity from the time of their first treatment with elimusertib (BAY 1895344) and gemcitabine.

Evaluable for Objective Response. Only those patients who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

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

12.3 Disease Parameters

Measurable Disease. Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥20 mm (≥2 cm) by chest x-ray or as ≥10 mm (≥1 cm) with CT scan, MRI, or calipers by clinical exam. All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Malignant Lymph Nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm (≥1.5 cm) in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm [0.5 cm]). At baseline and in follow-up, only the short axis will be measured and followed.

Non-Measurable Disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm [<1 cm] or pathological lymph nodes with ≥10 to <15 mm [≥1 to <1.5 cm] short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

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

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

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

Non-Target Lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

12.4 Methods for Evaluation of Measurable Disease

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

The same method of assessment and the same technique should be used to characterize each

identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be

imaged but are assessable by clinical exam.

Clinical Lesions. Clinical lesions will only be considered measurable when they are superficial (*e.g.*, skin nodules and palpable lymph nodes) and ≥10 mm (≥1 cm) diameter as assessed using calipers (*e.g.*, skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

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

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

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

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

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

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

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

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

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

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

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

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

12.5 Response Criteria

12.5.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm (<1 cm).

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

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm (0.5 cm). (Note: the appearance of one or more new lesions is also considered progressions).

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

12.5.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm [<1 cm] short axis).

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

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

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

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

12.5.3 Evaluation of Best Overall Response

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

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

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

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

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

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

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

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

12.5.4 Duration of Response

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

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

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

13. STUDY OVERSIGHT AND DATA REPORTING /REGULATORYREQUIREMENTS

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.

13.2 Data Reporting

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

- A valid CTEP-IAM account and linked ID.me account (ID.me accounts are required for all newly created CTEP-IAM accounts and by July 1, 2023 for all users), and
- Assigned Rave role on the LPO or PO roster at the enrolling site of: Rave CRA, Rave

Read Only, Rave CRA (LabAdmin), Rave SLA, or Rave Investigator.

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

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 the Regulatory application all persons with Rave roles assigned on the appropriate roster will be sent a study invitation email from iMedidata. To accept the invitation, site staff must either click on the link in the email or log in to iMedidata via the CTSU members' website under *Data Management* > *Rave Home* and click to accept the invitation in the Tasks pane located in the upper right-corner of the iMedidata screen. Site staff will not be able to access the study in Rave until all required Medidata and study specific trainings are completed. Trainings will be in the form of electronic learnings (eLearnings) and can be accessed by clicking on the eLearning link in the Tasks pane located in the upper right corner of the iMedidata screen. If an eLearning is required for a study and has not yet been taken, the link to the eLearning will appear under the study name in the Studies pane located in the center of the iMedidata screen; once the successful completion of the eLearning has been recorded, access to the study in Rave will be granted, and a Rave EDC link will replace the eLearning link under the study name.

Site staff who have not previously activated their iMedidata/Rave account at the time of initial site registration approval for the study in the regulatory application 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 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.

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 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, DPQ Form Status and the DQP Reports modules are available to access details and reports of unanswered queries, delinquent forms, forms with current status and timeliness reports. Site staff should review the DQP modules on a regular basis to manage specified queries and delinquent forms.

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

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

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

13.4 CTEP Multicenter Guidelines

N/A

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 Incidental/Secondary Findings Disclosure Procedure

Given the potential clinical implications conferred by detecting a germline and/or somatic mutation in one of the proven cancer susceptibility genes, this protocol will use the following disclosure procedure, consistent with the recommendations of the American College of Medical and Genomics (ACMG) (Green *et al*., 2013 and Kalia *et al.,* 2016):

The NCLN Genetics Laboratory will review the mutations/variants once at the time of initial specimen evaluation according to the most recent version of the ACMG guidance on variants. The NCLN Genetics Laboratory will not re-review all specimens received if a new version of the ACMG guidance is published after the initial review.

For each participant with a pathogenic or likely pathogenic germline and/or somatic variant detected in the WES of blood (as defined in the ACMG guidance), the NCLN Genetics Laboratory will report to the Program Director or Scientific Officer the UPID and variant(s) identified. The Program Director or Scientific Officer will contact Theradex to obtain the name of the protocol, investigator treating the patient, and the Principal Investigator of the grant. The treating physician will be contacted by phone and in writing to ask the patient whether he or she is interested in learning more about the finding.

If the patient wants to know more, the physician should contact the Program Director for more information about the mutation/variant. The treating physician and a medical genetics counselor should meet with the patient to discuss the importance and meaning of the finding, but not the finding itself, and notify the patient that this research finding must be confirmed by Sanger sequencing at the patient's/patient insurer's expense in a Clinical Laboratory Improvement Amendments (CLIA)-approved laboratory. The treating physician and genetic counselor should inform the patient of the confirmed result and its meaning and significance to the patient. If desired, the patient may elect to undergo genetic counseling and confirmatory CLIA-approved clinical testing on his or her own. Neither the research laboratory nor the National Cancer Institute will be responsible for the costs incurred for any confirmatory genetic testing or counseling.

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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

CTEP-assigned Protocol #_10403

Local Protocol # _____

PATIENT'S MEDICATION DIARY – ONCE A DAY DOSING

Today's date_____**Agent**Elimusertib (BAY 1895344)_____

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

INSTRUCTIONS TO THE PATIENT:

1. Complete one form for each cycle of treatment.
2. You will take____of____mg and____of____mg of elimusertib (BAY 1895344) tablet(s) **as directed**, one (1) hour before or two (2) hours after a meal with approximately 240 mL (approximately 8 ounces) of water.
3. **Take elimusertib (BAY1895344) tablets only on days 2-3 and days 9-10**
4. Record the date and the time that you took the tablets.
5. If you have any comments or notice any side effects, please record them in the Comments column.
6. If you vomit after taking elimusertib (BAY 1895344), do not retake the dose. Take the next dose as originally scheduled.
7. You will take elimusertib (BAY 1895344) as prescribed (+/- 3 hours). If you miss a dose, skip that dose and resume study medication at the next scheduled dose.
8. Avoid prolonged exposure to the sun and wear protective clothing and sunscreen when out in the sun.
9. Please bring this form and your bottles of elimusertib (BAY 1895344) tablets when you return for each appointment. Elimusertib (BAY 1895344)tablets are dispensed in original 18 count containers and you will have more tablets than you need.
10. Do not crush, chew, or dissolve elimusertib (BAY 1895344) in water
11. Do not to take elimusertib (BAY 1895344) prior to visit on D1 and D8. You will also need to wait to dose in clinic on D2 of Cycle 1 only.
12. Do not consume grapefruit or Seville oranges
13. Please take elimusertib (BAY 1895344) as directed

Day	Date	Time of dose	Number of 10mg tablets taken	Number of 20mg tablets taken	Comments
1		X	X	X	X
2					
3					
4		X	X	X	X
5		X	X	X	X
6		X	X	X	X
7		X	X	X	X
8		X	X	X	X
9					
10					
11		X	X	X	X
12		X	X	X	X
13		X	X	X	X
14		X	X	X	X
15		X	X	X	X
16		X	X	X	X
17		X	X	X	X
18		X	X	X	X
19		X	X	X	X
20		X	X	X	X
21		X	X	X	X

Patient's signature

_____

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 tablets taken this month

   _____
5. Physician/Nurse/Data Manager's Signature

   _____

CTEP-assigned Protocol #_10403
Local Protocol #_____

PATIENT'S MEDICATION DIARY - TWICE A DAY DOSING

Today's date_____**Agent**_____Elimusertib (BAY 1895344)_____

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

1. INSTRUCTIONS TO THE PATIENT:Complete one form for each cycle of treatment.
2. You will take_____of____mg and____of____mg of elimusertib (BAY 1895344) tablet(s) **as directed**, one (1) hour before or two (2) hours after a meal with approximately 240 mL (approximately 8 ounces) of water.
3. **Take elimusertib (BAY 1895344) tablets only on days 2-3 and days 9-10**
4. Record the date and the time that you took the tablets.
5. If you have any comments or notice any side effects, please record them in the Comments column.
6. If you vomit after taking elimusertib (BAY 1895344), do not retake the dose. Take the next dose as originally scheduled.
7. You will take elimusertib (BAY 1895344) as prescribed (+/- 3 hours). If you miss a dose, skip that dose and resume study medication at the next scheduled dose.
8. Avoid prolonged exposure to the sun and wear protective clothing and sunscreen when out in the sun.
9. Please bring this form and your bottles of elimusertib (BAY 1895344) tablets when you return for each appointment. Elimusertib (BAY 1895344) tablets are dispensed in original 18 count containers and you will have more tablets than you need.
10. Do not crush, chew, or dissolve elimusertib (BAY 1895344) in water
11. Do not to take elimusertib (BAY 1895344) prior to visit on D1 and D8. You will also need to wait to dose in clinic on D2 of Cycle 1 only.
12. Do not consume grapefruit or Seville oranges
13. Please take elimusertib (BAY 1895344) as directed

Day	Date	Time of morning dose	Number of 10mg tablets taken	Number of 20mg tablets taken	Time of evening dose	Number of 10mg tablets taken	Number of 20mg tablets taken	Comments
1		X	X	X	X	X	X	X
2								
3								
4		X	X	X	X	X	X	X
5		X	X	X	X	X	X	X
6		X	X	X	X	X	X	X
7		X	X	X	X	X	X	X
8		X	X	X	X	X	X	X
9								
10								
11		X	X	X	X	X	X	X
12		X	X	X	X	X	X	X
13		X	X	X	X	X	X	X
14		X	X	X	X	X	X	X
15		X	X	X	X	X	X	X
16		X	X	X	X	X	X	X
17		X	X	X	X	X	X	X
18		X	X	X	X	X	X	X
19		X	X	X	X	X	X	X
20		X	X	X	X	X	X	X
21		X	X	X	X	X	X	X

Patient's signature

_____

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 tablets taken this month

   _____
5. Physician/Nurse/Data Manager's Signature

   _____

APPENDIX D PATIENT DRUG INTERACTIONS HANDOUT AND WALLETCARD

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

Patient Name:	**Diagnosis:**	**Trial #:**	
Study Doctor:	**Study Doctor Phone #:**	**Study Drug(s):**	Elimusertib (BAY 1895344) Gemcitabine

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.

These are the things that your healthcare providers need to know:
Elimusertib (BAY 1895344) interacts with certain specific enzyme(s) in your liver or other tissues like the gut, and certain transport proteins that help move drugs in and out of cell.

	Explanation
CYP isoenzymes	The enzymes in question are **CYP3A4, 2C8, 2C9, and 2C19**. Elimusertib (BAY 1895344) is broken down by CYP3A4 and may be affected by other drugs that inhibit or induce this enzyme. Strong inhibitors and inducers of CYP3A4 should be avoided. Elimusertib (BAY 1895344) weakly to moderately inhibits CYP3A4, CYP2C8 2C9, and 2C19. Elimusertib (BAY 1895344) induces CYP3A4 and2C19. Substrates of CYP3A4 with narrow therapeutic window should be avoided. Other drugs that are broken down by CYP2C8, 2C9, and 2C19 may be affected when used at the same time.
Transport proteins	The proteins in question are **P-gp, BCRP, OATP1B1, and OATP1B3**. Elimusertib (BAY 1895344) inhibits these transport proteins and may affect other drugs that require them to move in and out of the cells.

These are the things that you need to know:

The study drug elimusertib (BAY 1895344) may interact with other drugs which can cause side effects. For this reason, it is very important to tell your doctors about all your medicines, including: (a) medicines you are taking before this clinical trial, (b) medicines you start or stop taking during this study, (c) medicines you buy without a prescription (over-the-counter remedy), (d) herbals or supplements (e.g. St. John's Wort). It is helpful to bring your medication bottles or an updated medication list with you.

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 inhibitors and inducers of CYP3A4, substrates of CYP3A4 with narrow therapeutic window, substrates of CYP2C8, 2C9, 2C19, BCRP, P-gp, OATP1B1, and OATP1B3.

- 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.
- Make sure your doctor knows to avoid certain prescription medications.
- No grapefruit juice, Seville oranges, or grapefruit can be consumed while on elimusertib (BAY 1895344).
- Antacids, H2 receptor antagonists, and proton pump inhibitors should be used with caution.
- Avoid prolonged exposure to the sun and wear protective clothing and sunscreen when out in the sun.
- 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.

Version Nov/2019

(Next page: Patient Drug Interaction Wallet Card)

PATIENT DRUG INTERACTION WALLET CARD

NIH NATIONAL CANCER INSTITUTE **EMERGENCY INFORMATION**	NIH NATIONAL CANCER INSTITUTE	NIH NATIONAL CANCER INSTITUTE	NIH NATIONAL CANCER INSTITUTE **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** Elimusertib (BAY 1895344) interacts with specific liver enzymes called CYP3A4, 2C8, 2C9, and 2C19 and transport proteins BCRP, P-gp, OATP1B1, and OATP1B3 and must be used very carefully with other medicines that interact with these enzymes or transporters.	
Patient Name: **Diagnosis:** **Study Doctor:** **Study Doctor Phone #:** **NCI Trial #:** **Study Drug(S):** Elimusertib (BAY 1895433), **Gemcitabine**	**Use caution and avoid the following:** • No grapefruit juice, Seville oranges, or grapefruit can be consumed while on elimusertib (BAY 1895344). • Antacids, H2 receptor antagonists, and proton pump inhibitors should be used with caution. • Avoid prolonged exposure to the sun and wear protective clothing	Your healthcare providers should be aware of any medicines that are strong inhibitors or inducers of CYP3A4 and substrates of CYP3A4 with narrow therapeutic window, which should be avoided. Use caution with substrates of CYP2C8, 2C9, 2C19, BCRP, P-gp, OATP1B1, and OATP1B3. **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 Nov/2019	
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

APPENDIX E 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. IR co-investigators are encouraged to apply this pre-biopsy scoring and correlation system to assist in the determination of biopsy appropriateness.

- IR 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 F TISSUE BIOPSY VERIFICATION

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

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 EET Biobank. A completed copy of this appendix (i.e., Tissue Biopsy Verification) must also be submitted to the EET Biobank.

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

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 Metastatic

Time point (circle one): Baseline C1D9 Disease Progression

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.

Version: 1
Effective Date: 9/2019

APPENDIX G SPECIMEN COLLECTION LOG ORGANOID CULTURES(OVARIAN CANCER)

Sample identification:
Specimen collection location:
Specimen collection date and time: Date: _____ Time:_____AM/PM
Additional notes on specimen collection: (optional)

APPENDIX H NCLN PHARMACODYNAMICS LABORATORY FROZENBIOPSY COLLECTION PROCEDURE

DCTD Standard Operating Procedures (SOP)

Title:	Tumor Frozen Needle Biopsy Specimen Collection, Handling and Shipment to EET Biobank				Page 1 of 13
Doc. #:	SOP340567	Revision:	-	Effective Date:	10/08/2021

Laboratory of Human Toxicology & Pharmacology

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

Frederick National Laboratory for Cancer Research

Technical Reviewer: Li Li Date: Li Li -S (Affiliate) Digitally signed by Li Li -S (Affiliate) Date: 2021.10.08 09:55:19 -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: 2021.10.13 11:00:43 -04'00'

LHTP Approval: Ralph E. Parchment Date: Ralph E. Parchment -S (Affiliate) Digitally signed by Ralph E. Parchment -S (Affiliate) Date: 2021.11.30 11:28:47 -05'00'

DCTD OD Approval: Toby Hecht Date: Toby T. Hecht -S Digitally signed by Toby T. Hecht -S Date: 2021.12.06 11:58:47 -05'00'

Change History

Revision	Approval Date	Description	Originator	Approval
--	10/08/2021	New Document	LL/RA/KFG	KFG

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.

TABLE OF CONTENTS

Title:	Tumor Frozen Needle Biopsy Specimen Collection, Handling and Shipment to EET Biobank				Page 3 of 13
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1.0 PURPOSE

Standardize the method for collecting, handling, and shipping frozen needle tumor biopsies toEET Biobank to enable measurement of pharmacodynamic (PD) markers following treatmentwith anticancer agents.

2.0 SCOPE

This procedure applies to all personnel involved in the collection and handling of frozen needletumor 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
EET Biobank	=	NCI Early-Phase and Experimental Clinical Trials Biospecimen Bank, also referred to as the Nationwide Biorepository or ETCTN Biorepository
FNLCR	=	Frederick National Laboratory for Cancer Research
ID	=	Identification / Identifier
IQC	=	Internal Quality Control
LHTP	=	Laboratory of Human Toxicology and Pharmacology
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 fromthis procedure allow retrospective identification of artifactual changes in biomarker readout andincreases 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 handleclinical samples.

Certified Assay Operator and/or an assay operator and/or PK/PD Support Lab personnel may PK/PD Support Lab Personnel be a Laboratory Technician/Technologist, Research Associate, or Laboratory Scientist who has been trained by DCTD personnel on this SOP. Working under the guidance of the Laboratory Director/Supervisor, this person performs laboratory procedures and examinations in accordance with thecurrent SOP(s), as well as any other procedures conducted by alaboratory, 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 assay operator running the SOP has sufficient experience to handle and analyze clinical samples. To become proficient with this SOP, sites are highly encouraged to reach out to NCI_PD_Support@mail.nih.gov for additional training materials.

5.2 It is the responsibility of the assay operator to confirm scheduled specimen collection time points, pre-print all labels, request access to **NCI Medidata Rave** (ETCTN Specimen Tracking System), check documentation for accuracy, request sample shipping kits from the EET Biobank and verify that the required collection tubes, supplies, and equipment are available for successful collection and handling of biopsy samples.

5.3 It is the responsibility of the assay operator to conduct the specimen collection and handling procedures following this SOP and complete the required tasks and associated documentation. The Biopsy Collection Record (Appendix 1) must be completed for each patient sample collection and filed with the study patient's other records.

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 the 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 -80°C freezer (or colder)

6.10 Specimen shipping kit from EET Biobank

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7.0 OPERATING PROCEDURES

7.1 This SOP uses **NCI Medidata Rave** for sample tracking, please review the following training videos for **NCI Medidata Rave** before you start:

7.1.1 General RAVE training:
https://www.youtube.com/watch?app=desktop&v=ZRX0lSqs5zo

7.1.2 Label Printing training:
https://www.youtube.com/watch?app=desktop&v=9_Q6_k-KHHs

7.2 Sample Shipping Kits

Sample shipping kits should be requested prior to enrolling the first biopsy patient from EET Biobank by emailing BPCBank@nationwidechildrens.org. For current customers, the kits can be requested through the EET Biobank (kit management system: https://kits.bpc-apps.nchri.org/Auth/Login?ReturnUrl=%2f. Please allow 5-7 business days for kit shipment.

7.3 Labels

7.3.1 Prepare enough pre-printed specimen labels in **NCI Medidata Rave** by following steps 7.3.1.1- 7.3.1.5:

7.3.1.1 Log into **NCI Medidata Rave** and go to **Enrollment** folder and confirm the **Histology and Disease** form is complete.

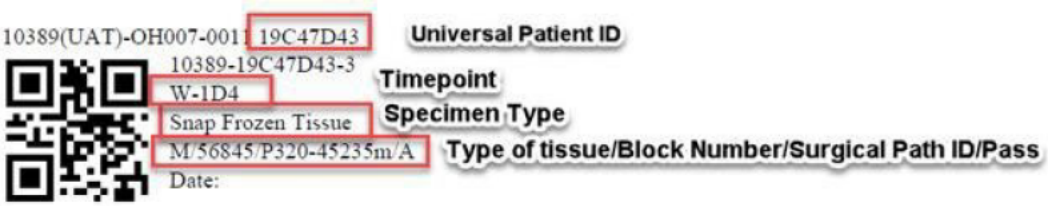
7.3.1.2 Go to **All Specimens** folder.

7.3.1.3 Complete the **Specimen Consent** form.

7.3.1.4 Complete the **Specimen Tracking Enrollment** form for each specimen.

7.3.1.5 Complete the **Print Labels** form. Labels will be sent to user's email address. For tissue specimens, apply appropriately coded label to each pass of the biopsy (see below).

Note: Five labels will be printed by default when you enter "**1**" in the "**How many labels are needed**" field. The first four will be designated with A, B, C and D to represent different passes of the biopsy procedure. Please use those accurately to label the specimens; pass A should be for the first pass, B for the second etc. The fifth label will have no pass designation and can be used on reports to be uploaded into RAVE. See an example of pre-printed label for frozen tissue biopsy below.



7.4 Tumor Needle Biopsy Collection and Handling

7.4.1 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 frozen specimens from the procedure area to the laboratory, where they will be placed into storage at -80ºC (or colder).

7.4.2 Prior to biopsy, the lesion should be assessed as to whether or not the biopsy should be performed and yield a successful outcome. Fill out the **Pre-Biopsy Lesion Score** form in **NCI Medidata Rave** using inputs from interventional radiologists and/or oncologists.

7.4.3 Bring all necessary lab supplies to the biopsy collection site, including: disposable tweezers, a minimum of four 1.5-mL Sarstedt tubes pre-cooled on liquid nitrogen or dry ice/ethanol in an insulated bucket (Sarstedt tubes will be provided in the sample shipping kit from EET Biobank; please use one tube for each whole biopsy core), the label with no pass designation to give to the research nurse for the patient record, and a printout of Appendix 1.

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

7.4.4 The total time elapsed between biopsy collection and placement into the pre-chilled tube is of **key importance** to biomarker analysis; this time should be documented in **NCI Medidata Rave** for each biopsy pass. **It is important to note that all 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 at this point (or note the time in Appendix 1) and immediately walk the slide to the sample preparation table for transfer to the pre-chilled Sarstedt tube.

7.4.5 Immediately snap freeze the biopsy by placing the tube in liquid nitrogen or a dry ice/ethanol bath (stop the stopwatch at this point). **Note:** DO NOT let the tubes tip over in the liquid nitrogen or dry ice/ethanol bath.

7.4.6 Calculate the total time elapsed from biopsy collection to biopsy freezing and record the total number of **minutes and seconds** (Appendix 1).

7.4.7 Note the specific needle type used and location of each biopsy pass collected (*e.g.*, spleen, large left upper quadrant splenic mass) (Appendix 1).

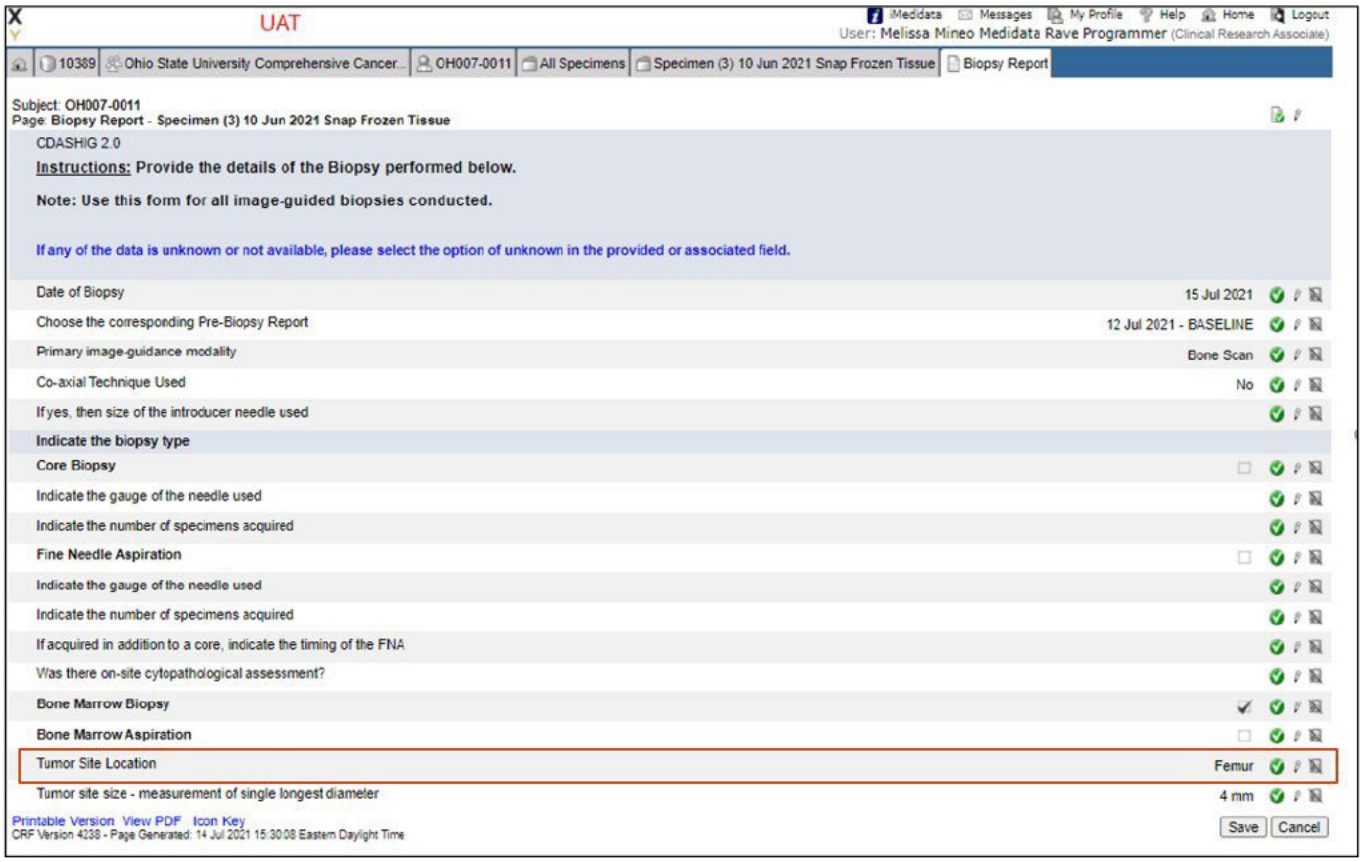
7.4.8 Note the protocol biopsy timepoint in Appendix 1.

7.4.9 Return to the sample processing laboratory and transfer the frozen biopsy specimen(s) to -80°C (or colder) for storage until shipment to the EET Biobank.

7.4.10 After biopsy collection, complete sample tracking documentation in **NCI Medidata Rave** according to notes recorded in Appendix 1.

7.4.11 Fill out **Biopsy Report** form in the **All Specimen** folder.

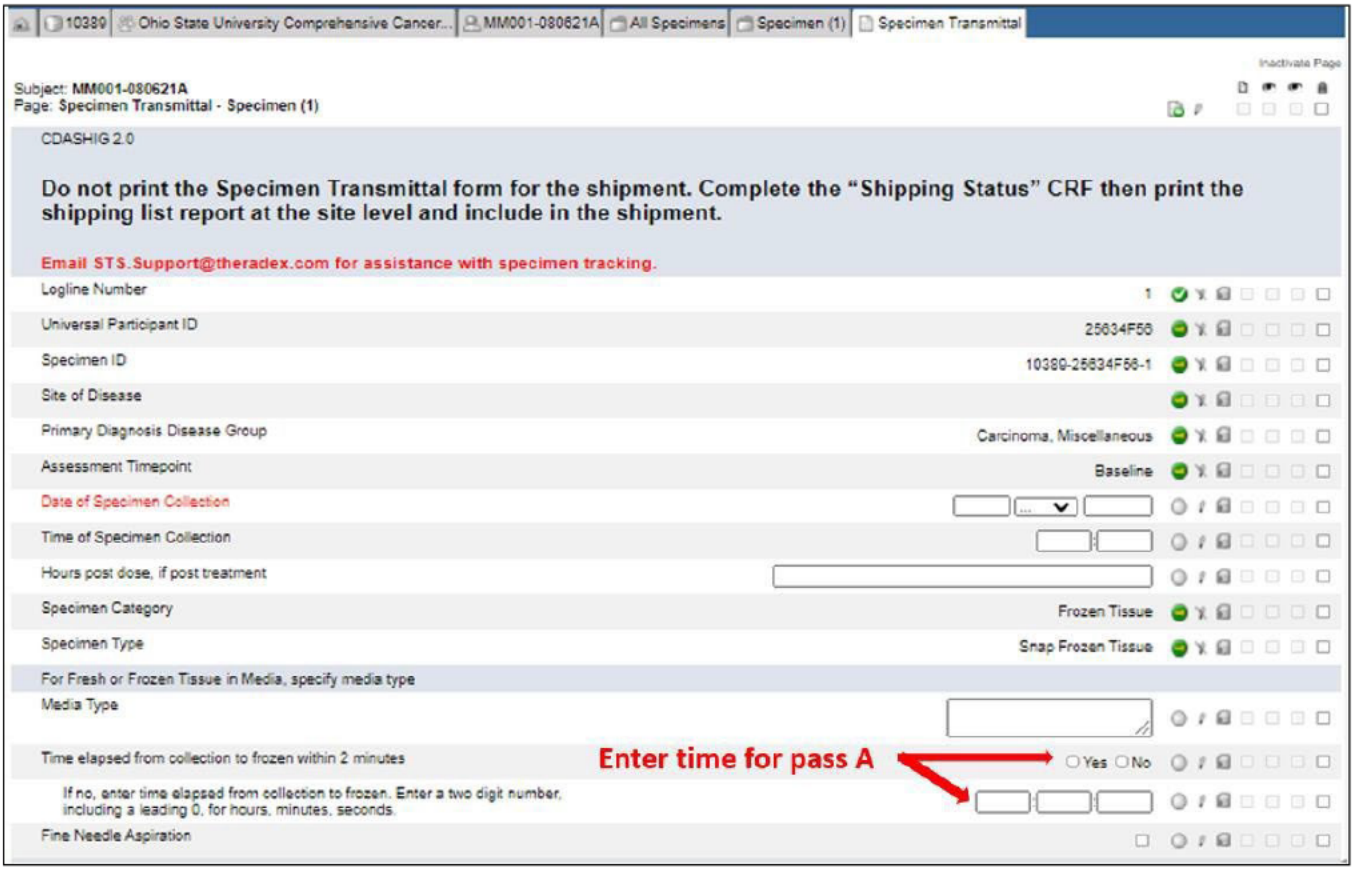
Note: It is very important to record the site of the biopsy to the **Tumor SiteLocation** field as shown below.



7.4.12 Complete the **Specimen Transmittal** form in the All Specimen folder.

Note: It is important to fill out the time from collection to frozen for each pass inthe **Specimen Transmittal** form by following the instructions below.

Pass A time will be recorded in the appropriate fields as shown below:



Times elapsed for passes B, C and D will be recorded in the **Comment** field nearthe bottom of the Specimen Transmittal form as shown below. Biopsy passes notcollected will also be recorded in the **Comment** field as shown

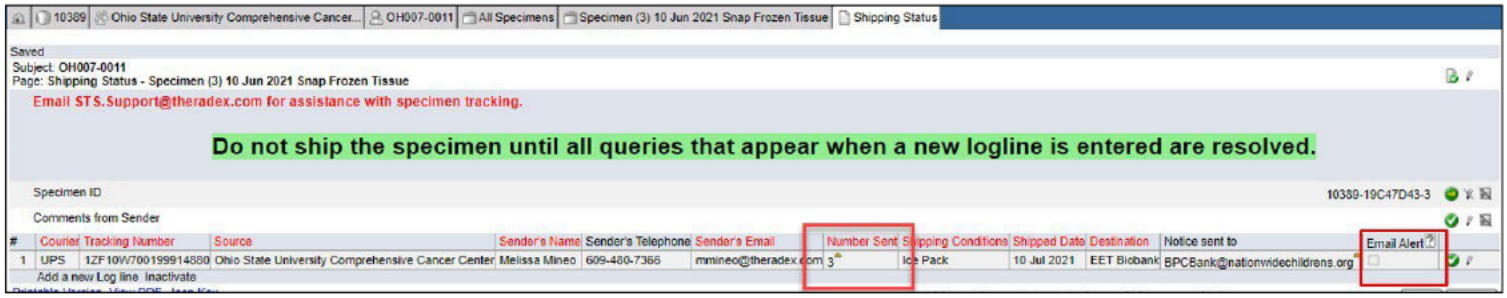
8.0 SHIP TO EET BIOBANK

8.1 When specimens are ready to be shipped, complete shipment documentation in **NCI Medidata Rave.**

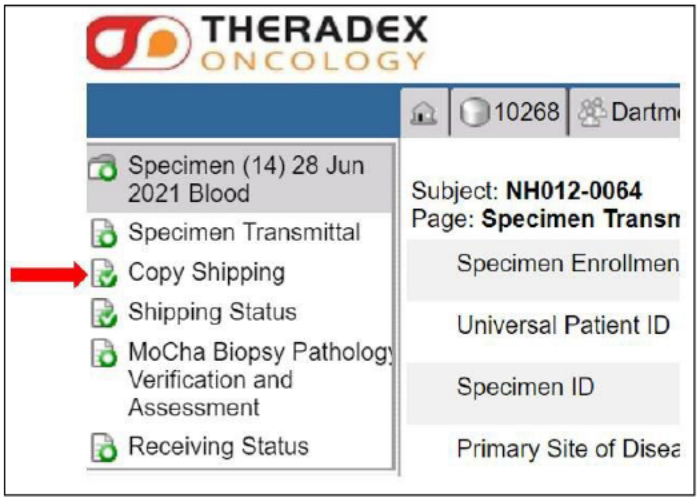
8.1.1 Complete the **Shipping Status** form.

8.1.1.1 Each field in the **Shipping Status** form should be completed as shown below and **Number Sent** (circled below) should equal the number of biopsy passes in the shipment.

8.1.1.2 **Email Alert** (circled below) is only checked for the last specimen in a shipment if multiple specimens are shipped together.

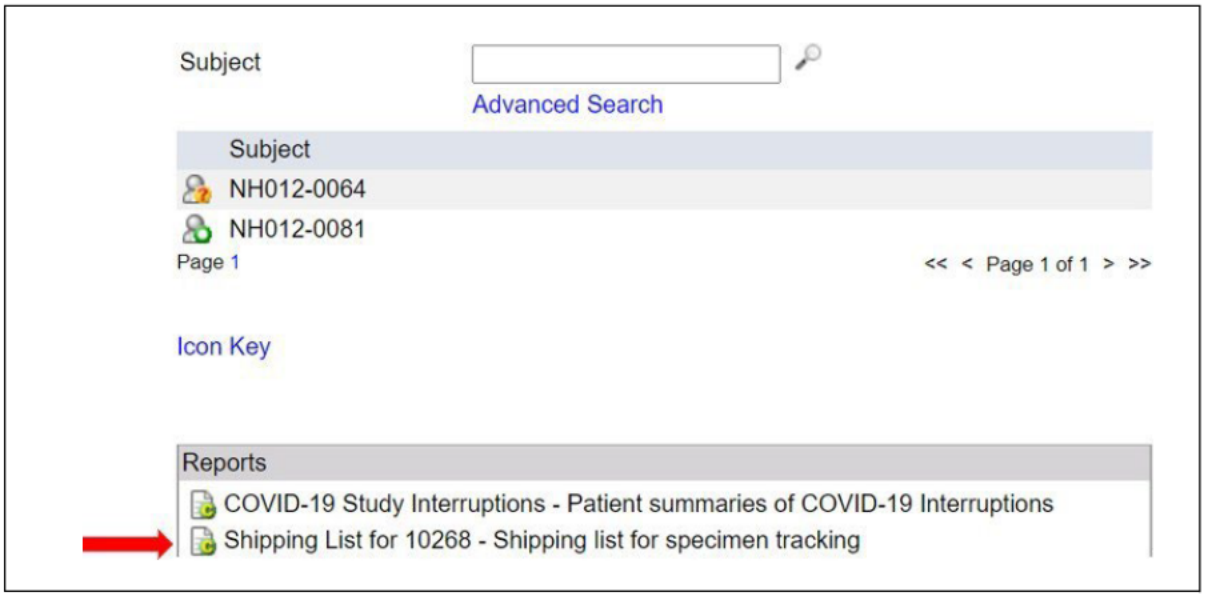


8.1.2 If there are other specimens to be shipped with the frozen biopsies, use the **Copy Shipping** utility form (shown below) in the other specimens' folder.



8.1.3 Print the **Shipping List** report and place it in the box with the specimens.

8.1.3.1 The **Shipping List** report is found in the report panel at the bottom of the window at the site level (an example shown below) since specimens from multiple patients can be included in a single shipment.



8.1.3.2 Shipment should include a hard copy (printed copy) of the **Shipping List**. An example is shown below.

Shipping List

Protocol: 10389 - UAT
Site: Ohio State University Comprehensive Cancer Center
Shipping Date: 10 Jul 2021
Tracking Number: 1ZF10W700199914880

Contact Info: Melissa Mineo
609-480-7366
mmineo@theradex.com

Please include a hardcopy of the pathology and any other relevant report in the shipment.

Protocol-Patient ID / Universal Pat. Id / Specimen ID	TimePoint	Category / Type	Samples Sent	Tissue	Sample Site	Collection Date/Time / Processed Date/Time / Frozen in 2 min/Elapsed	Comments
10389-OH007-0011 19C47D43 10389-19C47D43-1	Baseline	Blood Blood	1		General Blood Draw	21 Jun 202109:00 N/A	
10389-OH007-0011 19C47D43 10389-19C47D43-2	Archival	Formalin Fixed Paraffin Embedded Tissue FFPE Block	1	Metastatic	Esophagus	09 Jan 202115:00 N/A	
10389-OH007-0011 19C47D43 10389-19C47D43-3	Week -1 Day 4 (Expansion Cohort Only)	Frozen Tissue Snap Frozen Tissue	3	Metastatic	Esophagus	10 Jun 202111:10 N/A No/00:03:25	Pass B time to frozen: 02:20 ; Pass C time to frozen: 00:50

8.1.3.3 Shipment should also include a hard copy of the **TISSUE BIOPSY VERIFICATION** form found in the appendices of corresponding protocols.

8.2 Specimen shipment to EET Biobank

8.2.1 Follow the **Shipping Specimens from Clinic Site to the EET Biobank/ETCTN Biorepository** section of the clinical protocol for general instructions of sample shipment to EET Biobank.

8.2.2 Frozen biopsies should be shipped in kits provided by EET Biobank. The shipping container sent with kit contents should be used to ship specimens to EET Biobank. **Note:** It's important to include sufficient dry ice to keep the biopsy frozen for at least 96 hours.

8.2.3 Frozen specimens may be shipped on Monday through Thursday to the following address:

EET Biobank
The Research Institute at Nationwide Children's
Hospital700 Children's Drive, WA1340
Columbus, Ohio 43205
PH: (614) 722-2865
FAX: (614) 722-2897

Note: FedEx Priority Overnight service is the required shipping method. The EET Biobank FedEx account will not be provided to submitting institutions. Sites are responsible for all costs for shipments to the EET Biobank, so theovernight express shipment should be billed directly to the shipping institution/site.

8.3 Useful contacts for Specimen Collection, Handling and Shipment:

8.3.1 Send all questions related to this SOP or PD- assay support questions to: NCI_PD_Support@mail.nih.gov

8.3.2 Send all technical questions about the Specimen Tracking System to: STS.Support@theradex.com

8.3.3 EET Biobank queries (kit inquiries and sample shipping): BPCBank@nationwidechildrens.org

APPENDIX I: BIOPSY COLLECTION RECORD

Note: This document lists important information to be recorded during the biopsy collection process forlater documentation in **NCI Medidata Rave.** The completed document should be filed with the study patient's other records at a predetermined location according to local policy for managing clinical trial information. Please **do not** include the document in the shipment to EET Biobank.

Certified Assay Operator: _____

Facility/Clinic Collecting Specimens: _____

Clinical Protocol Number: _____

Patient ID: _____

1. Biopsy Collection Information:

Note: Information collected in the table below will be entered in Medidata RAVE.

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

	Pass A	**Pass B**	**Pass C**	**Pass D**
Specimen ID				
Protocol timepoint of biopsy (Cycle, Day, and Hours post dose, if post treatment)				
Needle type				
Site of biopsy (complete for all passes or note "same" for replicate cores)				
Required: Time elapsed from collection to placement in tube	min sec	min sec	min sec	min sec
Date biopsy collected				
Time biopsy collected	:	:	:	:
Time biopsy placed in tube	:	:	:	:

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2. Notes, including any deviations from the SOP:

APPENDIX J: Randomization Procedure

During dose escalation, when two dose levels are open, patients will be randomly assigned to the dose levels. The DFCI statistical team will perform the randomization that will assign patients to a specific dose level. The DFCI team will then notify CTEP and other study investigators of the patient dose level assignments.

The randomization will be based upon the table generated below. To ensure that the assignment is random we created a table with 36 rows that correspond to the patients that will be enrolled in the trial in order of enrollment. The fist column indicates the patient while the remaining indicates the dose levels in a random order. When the x-th patient has to be assigned to a dose level, we choose the first open dose level in its row, starting from the left.

Randomization Table:

	Dose Levels in random order																								
1	B11	B7	1	6	8	3	B10	B2	5	2	10	4	B3	B8	7	11	B12	12	B13	B4	B1	B9	B5	B6	9
2	B3	8	12	9	B7	B12	B8	B1	B11	3	B2	6	B6	4	2	1	5	11	10	B13	7	B9	B4	B10	B5
3	7	B12	B10	2	B2	B11	B3	12	3	B7	9	10	4	B4	B5	B6	B8	6	8	5	B1	1	11	B13	B9
4	2	10	B13	6	B11	3	4	7	5	12	B7	B5	11	B12	B10	1	B1	B8	B4	8	B6	B3	9	B9	B2
5	B7	9	12	6	7	B11	B4	B5	10	B12	B10	B13	B2	B1	B6	5	1	11	B9	B3	8	B8	2	4	3
6	11	B13	B6	B12	B4	3	B11	B5	1	6	B1	B9	4	B7	8	12	B3	B10	B2	5	2	10	B8	9	7
7	6	12	5	B13	4	2	B1	9	B7	B6	B9	B5	B12	8	B2	B11	1	B3	3	10	B8	7	B10	B4	11
8	B13	B9	4	12	B10	B2	B6	3	1	B1	2	B4	B5	6	5	10	11	8	B11	7	9	B7	B8	B12	B3
9	12	B11	7	3	B1	B4	B13	B3	8	B12	1	9	B8	4	B9	B5	6	5	B2	B7	B6	10	11	B10	2
10	B4	2	B12	B13	8	B3	6	12	B2	7	B7	9	B5	4	B1	10	B11	B6	3	B10	B9	11	5	1	B8
11	B8	6	5	11	4	7	1	B9	B5	8	B13	B1	B11	B10	B3	9	B2	B12	12	3	B4	B7	B6	2	10
12	B11	B2	B7	8	B3	B6	1	11	B12	5	12	4	10	B9	B8	9	2	B5	6	B1	B13	7	3	B10	B4
13	2	6	7	B6	9	12	10	4	B9	B13	B3	11	3	1	8	B2	B1	5	B11	B12	B4	B5	B8	B10	B7
14	B2	9	B8	7	B5	B3	3	6	2	4	12	B13	1	5	B10	8	B9	B12	B6	11	B11	B7	B1	B4	10
15	B13	5	B12	12	10	4	B10	8	B3	9	B7	B1	B5	1	7	11	B4	2	B9	B8	6	B2	3	B6	B11
16	5	10	2	7	B13	B6	4	B3	B1	3	6	9	B2	11	1	B9	8	B7	B11	B4	12	B5	B8	B12	B10
17	B7	B9	B6	3	1	12	B8	10	8	7	4	B10	B2	2	B11	B12	B3	B1	B13	6	9	11	B4	5	B5
18	B8	6	9	B11	B10	3	B13	B3	B4	2	7	B6	5	1	B1	4	10	8	B7	12	B9	B5	11	B2	B12
19	B12	9	B9	5	B3	6	8	1	B11	B6	B8	10	7	B4	4	B1	3	11	B7	12	B2	B5	B10	2	B13
20	B3	B11	9	12	B2	1	B6	B10	B13	4	10	5	B5	6	11	2	7	B7	3	B9	B4	B12	B8	8	B1
21	9	8	B3	B1	B13	6	4	B4	B2	B7	5	3	B6	11	1	B5	12	7	B8	B10	2	B11	10	B12	B9
22	3	B13	B5	7	B7	B1	6	B11	B2	B12	9	B8	B4	1	10	11	B6	2	B10	8	12	B3	4	5	B9
23	3	B11	7	10	6	B6	B8	5	11	B3	B12	B13	9	8	B4	4	B10	1	B7	B2	B5	B9	12	2	B1
24	4	B13	B10	B5	B2	5	B9	7	B1	11	B11	B6	3	2	10	B7	12	6	8	9	1	B8	B3	B4	B12
25	7	B9	B1	B3	B12	5	9	B5	4	3	B7	B8	B13	2	11	B10	B2	B6	1	6	10	B11	B4	8	12
26	B2	B12	8	B4	12	B8	9	B10	B1	B13	1	B11	B6	3	10	B3	7	2	B9	6	B5	B7	11	5	4
27	B12	B7	B11	B3	B4	B8	11	B13	6	8	4	B9	10	12	5	B2	B1	B5	B6	7	B10	9	3	1	2
28	B7	11	B6	2	B12	12	B4	10	B9	4	5	B5	B3	B1	B10	3	B2	B13	B11	1	B8	7	8	6	9
29	12	B6	2	B9	B5	B7	B3	4	7	B8	5	9	B12	3	8	11	6	10	B1	1	B11	B13	B10	B2	B4
30	9	B13	B8	B12	2	10	B10	B2	12	B11	5	B3	1	B6	B9	B5	8	7	3	11	B1	B4	4	B7	6
31	8	6	B2	B9	2	5	4	B1	12	11	B11	B12	10	7	B6	9	B7	B4	B10	B13	B3	B5	B8	1	3
32	B7	B11	8	4	11	6	B3	B8	7	B2	B5	B10	B13	1	2	9	B9	10	5	B1	B4	3	12	B6	B12
33	B11	B3	8	3	B9	12	B5	B10	B6	11	B12	5	9	B7	B8	B2	B1	4	B4	2	B13	1	7	6	10
34	10	11	B1	B9	9	B8	B2	B11	B13	B6	2	B10	1	B7	B12	8	B4	B3	3	6	7	5	12	4	B5
35	11	B3	B8	8	B4	B1	B12	1	B11	6	5	12	3	9	B7	B9	B6	B13	B5	B2	7	2	B10	10	4
36	B8	9	B5	5	B13	B12	3	B11	B6	B4	B9	B10	B3	B1	10	B2	12	2	1	7	B7	4	11	8	6