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TITLE: Phase 2 Single Arm Trial Testing the Azenosertib (ZN-c3) WEE1 inhibitor in combination with Gemcitabine in Second-Line Advanced Pancreatic Adenocarcinoma

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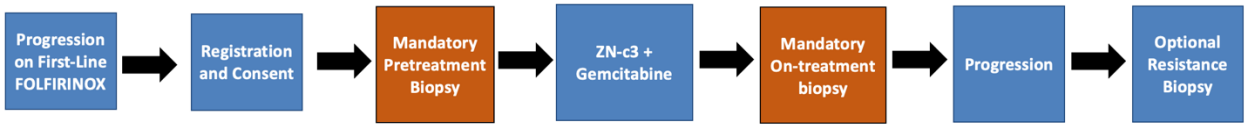
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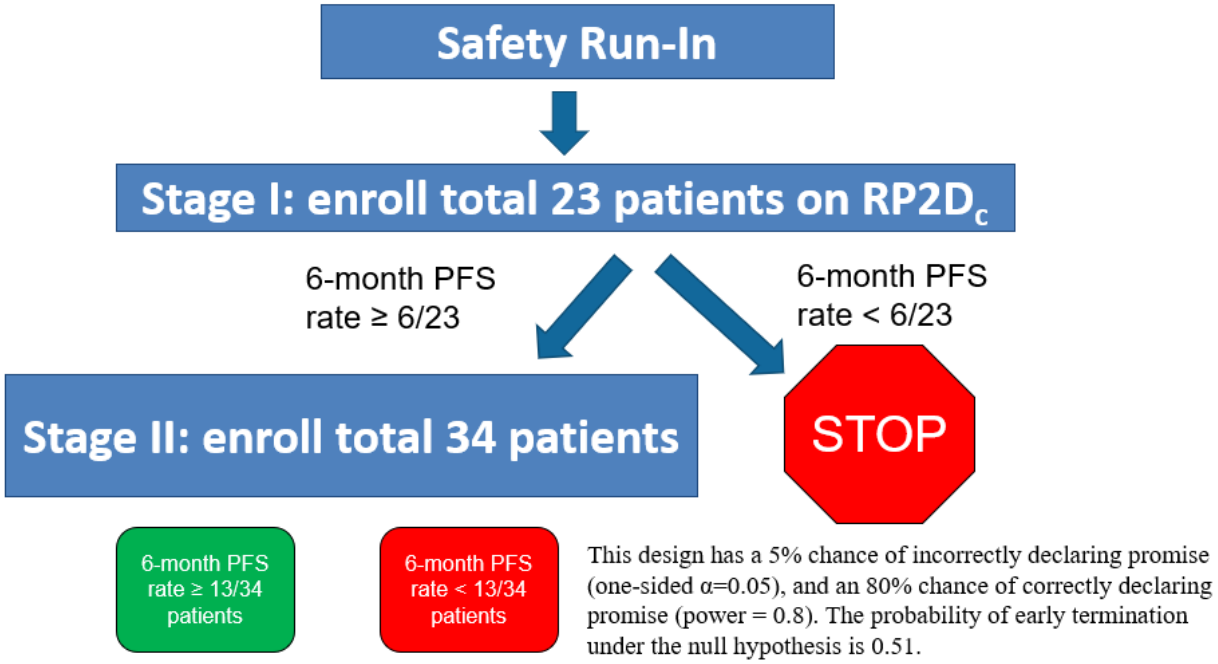
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SCHEMA





This design has a 5% chance of incorrectly declaring promise (one-sided α=0.05), and an 80% chance of correctly declaring promise (power = 0.8). The probability of early termination under the null hypothesis is 0.51.

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1. OBJECTIVES

1.1 Study Design

This is an open label, single arm phase 2 trial evaluating Azenosertib combined with gemcitabine in second-line advanced pancreatic cancer.

1.2 Primary Objectives

- To evaluate the efficacy of Azenosertib/gemcitabine, as measured by 6-month PFS among second-line advanced pancreatic cancer patients

1.3 Secondary Objectives

- To evaluate the overall survival (OS) and progression-free survival (PFS) of Azenosertib/gemcitabine in second-line advanced pancreatic cancer patients
- To evaluate of objective response rate per RECIST 1.1 criteria, disease control rate (defined as stable disease for ≥16 weeks, confirmed complete response, or confirmed partial response)
- To evaluate the safety and tolerability of this combination in platinum-resistant pancreatic cancer patients as assessed by the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.

1.4 Exploratory Objectives

- Explore biomarker indicators of target engagement, response, and resistance via analysis of tumor tissue and cell-free DNA (cfDNA)

2. BACKGROUND

2.1 Study Disease and Rationale

Pancreatic ductal adenocarcinoma (PDAC) is the fourth-leading cause of cancer-related death in the United States and is projected to become the second leading cause by 2030.[1] A major unmet need in PDAC is molecularly targeted treatment options for patients who are resistant to first-line platinum chemotherapy. While combined gemcitabine and nab-paclitaxel is commonly used after progression with first-line therapy, the efficacy in the second-line setting is fair, with recent phase clinical trial data demonstrating a median progression-free survival of 2.7-3.5 months and a median overall survival of 6.6-6.9 months.[2-4]

In recent years, preclinical work has identified that replicative stress is a potential PDAC vulnerability. Ordinarily, DNA replication is a tightly regulated cellular process designed to ensure fidelity of the genome. The regulatory processes that safeguard DNA replication become 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 cell cycle checkpoints resulting in prolongation of the S/G2 phase

of the cell cycle.[5,6] The uncontrolled cellular growth of cancerous cells, combined with inactivating mutations in many cell cycle checkpoint proteins, places them at risk for replicative stress. There is great interest to molecularly characterize replicative stress so that this potential vulnerability, which can lead to cell death through genomic catastrophe, can be exploited.[6] When a replication fork becomes unstable, because of factors such as DNA damage or lack of deoxyribonucleotide triphosphates (dNTPs), single stranded DNA in the stalled replication fork becomes coated with phosphorylated-RPA (pRPA).[7] The presence of pRPA leads to the activation of the regulatory kinases, ATR and its downstream effectors CHK1 and WEE1, which govern the cellular response to replicative stress. Unstable replication forks can be measured in the laboratory with DNA fiber assays while pRPA, phosphorylated ATR and CHK1 (pATR and pCHK1) can be assessed immunohistochemically.

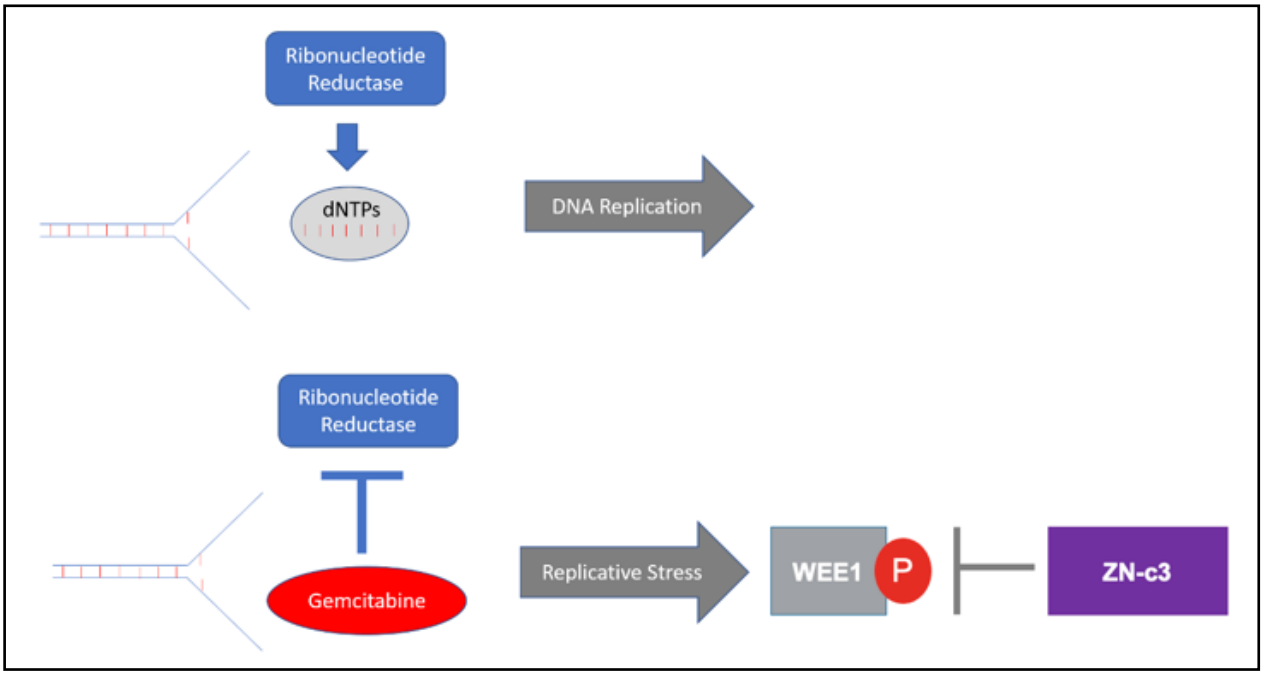


Figure 1. Schematic of clinical trials designed to exacerbate replication stress and prevent compensatory mechanisms for increased replication stress in PDAC. *KRAS mutation, CDKN2A/B* loss and *TP53* loss produce endogenous replicative stress in PDAC cells. This can be exacerbated by gemcitabine, a potent inhibitor of ribonucleotide reductase, that results in replication fork stalling, even at low dose. The WEE1 is critical for stabilizing stalled replication forks and the WEE1 kinase prevents premature mitotic entry until DNA replication is complete. Inhibition of WEE1 will further exacerbate replicative stress and induce lethality when combined with gemcitabine.

Therapies that inhibit cancer cells ability to compensate for replicative stress are particularly attractive in cancers that have genomic alterations that generate replicative stress by interfering with DNA damage repair (DDR) or driving rapid cell growth.[6] SLFN11 was discovered as a marker of resistance to DNA damaging cytotoxic chemotherapies, including topoisomerase inhibitors, platinum chemotherapies and alkylating agents [8,9]. Just as low levels of SLFN11 predicted resistance to DNA damaging cytotoxic chemotherapies, subsequent studies demonstrated that low levels of SLFN11 are also associated with resistance to PARP inhibitors.[10] While the exact function of SLFN11 is still being elucidated, mechanistic studies have revealed that SLFN11 is recruited to areas of DNA damage, through an interaction with Replication protein A (RPA), and inhibits the homologous recombination machinery from properly functioning.[11] Importantly, PARP inhibitor resistance caused by low SLFN11 levels can be overcome in *in vitro* models by combined ATR/PARP inhibition.[10] In addition to mutations that result in DDR

deficiency (*BRCA1, BRCA2, PALB2*, *ATM, CHEK2*, etc.), PDACs commonly harbor *KRAS* mutation, *CDKN2A/B* loss and P53 mutation.[12] Moreover, 5% and 3% of PDACs harbor *MYC* and *CCNE1* amplifications, respectively, which are also known to generate high levels of replicative stress.[13] We hypothesize that the high level of basal replicative stress in PDACs makes them susceptible to therapeutic strategies that both worsen replicative stress and inhibit pathways designed to compensate for replicative stress (**Figure 1**).[14-16]

The WEE1 kinase regulates the checkpoint governing the G2/M transition of the cell cycle. The G2/M checkpoint is critically important in malignancies like PDAC where over 75% of tumors have p53 pathway deficits.[13,17] Other investigators have demonstrated that gemcitabine combined with a WEE1 inhibitor (adavosertib) has anti-proliferative activity in some PDAC cell lines.[18] It was further demonstrated that *in vitro* and *in vivo* PDAC models were exquisitely sensitive to adavosertib-mediated WEE1 inhibition, though notably, P53 wild type tumors continued growing with exposure of gemcitabine/WEE1 inhibitor.[19,20] Others demonstrated that gemcitabine combined with adavosertib has preferential activity in homologous-recombination repair proficient cancers [18]. As a potential mechanism for the differential sensitivity of the gemcitabine/WEE1 inhibitor combination, it is possible that cells that do not stall in S phase are more likely to be forced into mitosis and undergo genomic catastrophe [18]. Consistent with these findings, Drean et al. demonstrated that *in vitro* and *in vivo* PDAC models with acquired resistance to PARP inhibitors were exquisitely sensitive to adavosertib-mediated WEE1 inhibition.[19] Further validation of the gemcitabine/WEE1 inhibitor combination was reported in a neoadjuvant PDAC trial where the combination was administered with radiation, demonstrating encouraging activity with median progression-free survival of 9.4 months and a median overall survival of 21.7 months, comparing favorably to those patients treated with other regimens in the unresectable setting.[21] Furthermore, data from a randomized phase II clinical trial in platinum resistant/refractory ovarian cancer demonstrated that patients treated with the gemcitabine/adavosertib combination had superior progression-free survival compared with patients on the gemcitabine/placebo arm.[22] However, notably, the most common grade 3/4 adverse events were cytopenias and fatigue highlighting a need for more tolerable WEE1 inhibitor/gemcitabine combination.

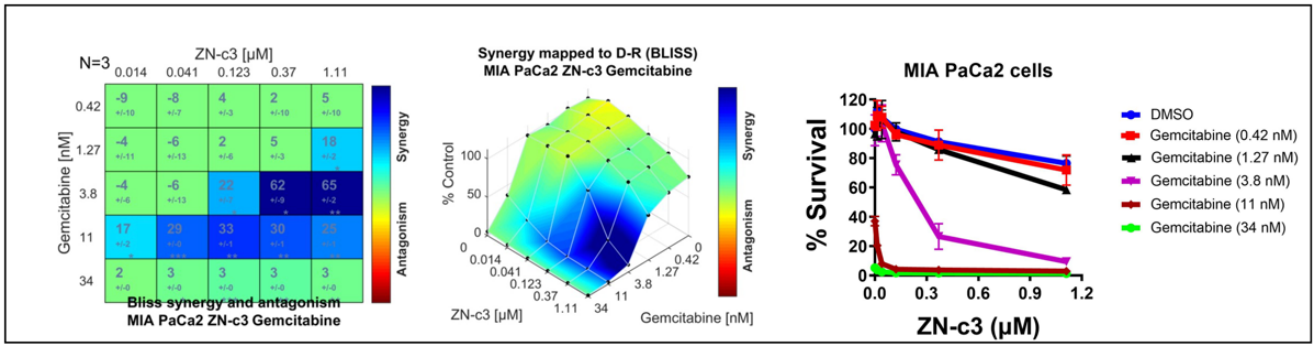


Figure 2. Combined gemcitabine and ZN-c3-mediated WEE1 inhibition is synergistic in the MIA PaCa2 PDAC cell line. The combination demonstrates synergism, demonstrated by survival and Bliss synergism plots in MIA PaCa2 PDAC cell line.

Azenosertib is an orally available, highly selective, nanomolar inhibitor of the WEE1 kinase that has more specific WEE1 kinase targeting with less effect on other kinases when compared to

adavosertib.[23] In the lab of Dr. Andy Aguirre, Azenosertib was combined with gemcitabine in PDAC cell lines demonstrating synergistic cytotoxicity based on the Bliss synergy scores (**Figure 2**). In preliminary phase 1 cohorts, Azenosertib was well-tolerated in combination with chemotherapy and exhibited lower hematologic toxicity and a better gastrointestinal tolerability profile in comparison to the Wee1 inhibitor class.[24] It is currently being evaluated in a phase 1 combination study in osteosarcoma and ovarian cancer. The RP2D has not been determined yet.

2.2 Azenosertib

Azenosertib is a novel, selective and orally bioavailable small molecule inhibitor of the WEE1 tyrosine kinase.

2.2.1 Clinical experience

Azenosertib is being evaluated in multiple expansion clinical trials. See the table below or refer to the Investigator's Brochure for more detailed information on the ongoing and completed studies using azenosertib.	**Study Number**	**N[a]**	**Study Description**	**Location**	**Status**
Tumor Type					
Advanced or metastatic solid tumors	ZN-c3-001[b]	274 (monotherapy)	Phase 1 open-label, multicenter, dose-escalation and expansion study of azenosertib monotherapy	US	Ongoing; Closed to enrollment
Ovarian, fallopian tube or primary peritoneal cancer	ZN-c3-002	160 (combination)	Phase 1b open-label, multicenter study of azenosertib in combination with PLD, carboplatin, paclitaxel,	Global	Ongoing

			gemcitabine, or bevacizumab		
Osteosarcoma	ZN-c3-003	31 (combination)	Phase 1/2 open-label, multicenter, dose-escalation and expansion study of azenosertib in combination with gemcitabine	Global	Completed
Uterine serous carcinoma	ZN-c3-004	76 (monotherapy)	Phase 2 open-label, multicenter study of azenosertib monotherapy	Global	Ongoing
Ovarian, fallopian tube, or primary peritoneal cancer	ZN-c3-005	108 (monotherapy; 6 in Part 1a; 102 in Part 1b)	Phase 2 open-label, multicenter study of azenosertib monotherapy	Global	Ongoing
Ovarian, fallopian tube, or primary peritoneal cancer	ZN-c3-006	117 (56 in combination; 61 in monotherapy)	Phase 1/2 open-label, multicenter, dose-escalation and expansion study of azenosertib in combination with niraparib and azenosertib monotherapy	Global	Ongoing; Closed to enrollment
Advanced or metastatic solid tumors	ZN-c3-008[b,c]	5 (monotherapy)	Phase 1 open-label, multicenter study to evaluate the effect of itraconazole on the pharmacokinetics of azenosertib (DDI study)	US	Ongoing
Metastatic colon cancer	ZN-c3-016	44 (combination)	Phase 1/2 study of azenosertib in combination with encorafenib and cetuximab in adults with	Global	Ongoing; Closed to enrollment

			metastatic colorectal cancer (joint study with Pfizer, Inc.)		
Acute myeloid leukemia	ZN-d5-004C	40 (13 in monotherapy; 27 in combination)	Phase 1/2 study of the BCL-2 inhibitor ZN-d5 and azenosertib in subjects with acute myeloid leukemia	US	Completed

Abbreviations: BCL-2, B-cell lymphoma 2; DDI, drug-drug interaction; PLD, pegylated liposomal doxorubicin; US, United States.
a Number of subjects treated as of a cutoff date of 30 September 2024.
b Three subjects who completed Study ZN-c3-008 were rolled over to Study ZN-c3-001 and are included in total numbers (N) for both studies.
c Study ZN-c3-008 is an ongoing DDI study with azenosertib and itraconazole; the data from this study are not included in the individual study summaries in the Investigator's Brochure but are included in the aggregate adverse events of special interest (AESI) data presented in Table 90 of the Investigator's Brochure.

2.2.2 Pharmacokinetics

Azenosertib potently inhibited the WEE1 kinase with an IC50 value of 3.8 nM and exhibited a high degree of kinase selectivity against a broad panel of 485 kinases. Only 4 other kinases, including PLK2, were observed to have more than 90% inhibition by Azenosertib at 1 μM. To further determine the potency of PLK2 inhibition by Azenosertib, an IC50 determination assay was used that showed Azenosertib inhibited PLK2 with an IC50 of 40 nM. Azenosertib inhibited the other 3 kinases with an IC50 of greater than 50 nM.

In cell proliferation inhibition assays, Azenosertib demonstrated *in vitro* concentration-dependent growth inhibition of NCI-H23 and A-427, both non-small cell lung cancer (NSCLC) cells. Azenosertib potently inhibited the proliferation of the A-427 cell line with an IC50 of 87.7 nM and the NCI-H23 cell line with an IC50 of 123 nM.

Please refer to the Azenosertib Investigator's Brochure (IB) for additional information.

2.2.3 Safety

The trials investigating its use in combination with PLD, carboplatin, paclitaxel, and gemcitabine were recently reported, but those studies are still ongoing. As of the presentation date of June 5, 2023, 51 subjects were evaluable for safety (i.e., received at least 1 dose of Azenosertib) in intermittent dosing. Adverse events (≥20% all Grade in any treatment group) were (all Grade/≥ Grade 3) [n(%)]: nausea 22 (43.1%)/3 (4.7%), neutropenia 28 (54.9%)/13 (25.5%), thrombocytopenia 24 (47.1%)/14 (27.5%), anemia 25 (49%)/8 (15.7%), diarrhea 19 (37.3%)/1 (2.0%), and fatigue 21 (41.2%)/5 (9.8%).[25] The study is ongoing including combination dose-finding (NCT04516447). Please refer to the Azenosertib Investigator's Brochure (IB) for

additional information.

As of the 30 September 2024 cutoff date, a total of 30 subjects died within 30 days after the last dose of azenosertib from a cause other than disease progression. Of these 30 deaths, 21 were deemed unrelated to azenosertib and 1 unknown cause of death was not determined. The remaining deaths were assessed by investigator as related to azenosertib and the causes included sepsis, neutropenia, acute respiratory distress syndrome, neurological disorders and fall. Please refer to the Azenosertib Investigator's Brochure (IB) for additional information.

2.3 Gemcitabine

Gemcitabine is currently FDA approved as a single agent and in combination with nab-paclitaxel for the treatment of advanced pancreatic adenocarcinoma. Commercially available gemcitabine will be administered according to institutional standards. Refer to the gemcitabine FDA package insert for additional information.

Gemcitabine kills cells undergoing DNA synthesis and blocks the progression of cells through the G1/S-phase boundary. Gemcitabine is metabolized by nucleoside kinases to diphosphate (dFdCDP) and triphosphate (dEdCTP) nucleosides. Gemcitabine diphosphate inhibits ribonucleotide reductase, an enzyme responsible for catalyzing the reactions that generate deoxynucleoside triphosphates for DNA synthesis, resulting in reductions in deoxynucleotide concentrations, including deoxycytidine triphosphate (dCTP). Gemcitabine triphosphate competes with dCTP for incorporation into DNA. The reduction in the intracellular concentration of dCTP by the action of the diphosphate enhances the incorporation of gemcitabine triphosphate into DNA (self-potentiation). After the gemcitabine nucleotide is incorporated into DNA, only one additional nucleotide is added to the growing DNA strands, which eventually results in the initiation of apoptotic cell death.

Please refer to the most recently approved local prescribing information for more details.

2.4 Azenosertib and Gemcitabine Combination

The MTD of Azenosertib in multiple-dose combination with gemcitabine has not been determined. It is currently being tested in osteosarcoma patients at 800 mg/m^2 on days 1 and 8 in a 21-day cycle and Azenosertib 150 mg daily on days 1-5, 8-12, and 15-19 and there have been zero DLTs in 6 patients. In gynecologic malignancy, it is being tested at 600 mg/m^2 on days 1 and 8 in a 21-day cycle and Azenosertib 200 mg on days 1-5, 8-12, and 15-19.

In consultation between the sponsor and the investigators, the single agent recommended Phase 2 dose (RP2D) was determined to be 300 mg QD continuous dosing based on the safety profile in Cycle 1 and subsequent cycles, the frequency of dose reductions and interruptions, and the pharmacokinetic profile.

Refer to the Azenosertib investigator brochure for additional information.

2.5 Correlative Studies Background

2.5.1 Whole Exome Sequencing (WES) and RNA Sequencing (RNAseq)

Each patient enrolled on the study will have whole transcriptome sequencing (WTS) by RNA-sequencing (RNAseq) performed on their biopsy to assess for baseline transcriptional signatures that may correlate with treatment response.[26] Multiple transcriptional subtypes have been described for pancreatic cancer, and these subtypes may have prognostic and predictive therapeutic implications.[27-32]

Whole exome sequencing (WES) will be performed on the biopsy and organoid specimens to assess for mutational signatures of DDR defects.[26] WES enables a comprehensive analysis of DNA mutation in human tumor samples.[33,34] The analyses will involve approximately 100 genes of interest in DNA repair. This will allow identification of further genomic variations or genomic signatures of altered DNA repair that may occur outside of the known genetic alterations that are identified via targeted genomic sequencing.

WES data will also be analyzed for base mutations, small insertions/deletions, and copy number alterations.

2.5.2 DNA Damage Repair Evaluation

Each tumor will be evaluated for RAD51 foci as a marker for functional HR defects, DNA replication fork stability assays, as well as ATM/ATR/CHK1/Geminin immunohistochemistry (IHC). Further evaluation may utilize the following pharmacodynamic markers: γH2AX foci/levels by western blot (WB) and IHC, cell-cycle profiling by fluorescence-activated cell sorting (FACS), pCHK1 (S296 and S345) levels by WB, pDNA-PK foci/levels by WB, pATM foci, pKAP1 levels by WB, pRPA levels by WB, CDC25A levels by WB, activation of CDK2 by WB, apoptosis markers by WB or FACS. Alternate or additional biomarkers may be explored as considered appropriate at the time of the analysis.

2.5.3 Organoid Culture Generation and Compound Sensitivity Testing

Treatment responsiveness likely represents a multi-factorial phenotype that is not well described by either genomic or transcriptomic assessment alone. We hypothesize that effective prediction of therapeutic responses in a clinically useful manner will require implementation of *ex vivo* functional profiling of PDAC tumor cell sensitivity.

Patient-derived pancreatic tumor organoids will be grown from tumor tissue collected from participants who enroll to the trial.[35] After several rounds of organoid expansion, sensitivity to therapeutic agents will be conducted. As part of this profiling, sensitivity to gemcitabine and Azenosertib will be performed. Additionally, the organoids will be tested for functional HR defects and may undergo sequencing. Results from the organoid testing will be compared to clinical outcome.

2.5.4 Cell-free DNA (cfDNA)

Serial blood sample collections will be processed for cfDNA. It has been recently demonstrated that the yield and purity of cfDNA is adequate for sequencing (25-30%) with variance in the quantity through the course of therapy. In addition to evaluating genomic alterations in DNA extracted from blood, the findings will be compared to parallel sequencing analysis done on the tissue biopsy from the same subjects to compare similarities and any potential differences. This analysis will help to determine whether cfDNA sequencing may be a possible substitute for tissue-based sequencing which would be critical, as tissue biopsies are both invasive and costly.

3. PARTICIPANT SELECTION

3.1 Inclusion Criteria

3.1.1 Participants must have a pathologically confirmed advanced pancreatic adenocarcinoma that is not curable with standard approaches based on the judgement of the treating investigator. Patients with metastatic pancreatic cancer and unresectable pancreatic cancer are eligible.

3.1.2 Patients must have progressed or not tolerated a platinum-based regimen prior to enrolling on the trial.

3.1.3 Patients must have received no more than 1 prior lines of platinum-based chemotherapy in the metastatic setting. Therapy given in the adjuvant or neoadjuvant setting is counted as a prior therapy if it occurred less than 6 months before cancer recurrence or progression.

3.1.4 Age ≥ 18 years. As no dosing or adverse event data are currently available in participants < 18 years of age, children and adolescents are excluded from this study.

3.1.5 ECOG performance status of 0 or 1 (see **Appendix A**) with no deterioration over the previous 2 weeks prior to day of first dosing (Cycle 1, Day 1).

3.1.6 Participants must have adequate organ and marrow function as defined below:

Absolute neutrophil count	≥ 1,500/mm^3
Platelets	≥ 100,000/mm^3 excluding measurements obtained within 3 days after transfusion of platelets
Hemoglobin	≥ 9 g/dL
Total bilirubin	≤ 1.5 × institutional upper limit of normal (ULN); or total bilirubin ≤3.0 x ULN with direct bilirubin WNL in patients with documented Gilbert's Syndrome.
Alanine aminotransferase (ALT) and aspartate aminotransferase (AST)	≤2.5 x institutional ULN; ≤ 5 × institutional ULN if liver metastases
Albumin	≥ 2.7 g/dL
Creatinine clearance (CrCl)	≥ 50 ml/min based on Cockroft-Gault method

3.1.7 Participants must have measurable disease by RECIST v. 1.1 criteria and be willing to undergo a pre-treatment and on-treatment tumor biopsy. The biopsy requirement can be waived only with approval from the sponsor-investigator.

3.1.8 For participants with evidence of chronic hepatitis B virus (HBV) infection, the HBV viral load must be undetectable on suppressive therapy, if indicated, unless there is a drug-drug interaction with study medication.

3.1.9 Patient has read and understands the informed consent form (ICF) and has been given written ICF prior to any study procedures which includes compliance with the requirements and restrictions listed in the ICF and in this protocol.

3.1.10 Female patients who are not of child-bearing potential* and women of childbearing potential who agree to use adequate contraceptive measures prior to the first dose and for 90 days after the last dose of Azenosertib, and who have a negative serum or urine pregnancy test within 14 days prior to the start of study treatment.

* Evidence of non-childbearing status, defined as below:
- Women who are surgically sterile (i.e., have undergone bilateral salpingectomy, bilateral oophorectomy, or complete hysterectomy).
- Age ≥50 years with any one or more of the conditions below:
  - Amenorrheic for 12 months or more following cessation of all hormonal replacement therapy
  - Had radiation-induced menopause with last menses >1 year ago
  - Had chemotherapy-induced menopause with last menses >1 year ago
- Age <50 if amenorrhoeic for 12 months or more following cessation of all hormonal replacement therapy and if luteinizing hormone and follicle-stimulating hormone levels are in the post-menopausal range per institutional standards of practice.

3.1.11 Male patients should be willing to abstain or use barrier contraception (i.e., condoms) for the duration of the study drug exposure and for 90 days after the last dose of Azenosertib after study treatment discontinuation.

3.1.12 Participants with a history of hepatitis C virus (HCV) infection must have been treated and cured. For participants with HCV infection who are currently on treatment, they are eligible if they have an undetectable HCV viral load, unless there is a drug-drug interaction with study medication.

3.2 Exclusion Criteria

3.2.1 Patients who have previously received a WEE1 inhibitor are not eligible.

3.2.2 Patients who received gemcitabine for incurable pancreatic cancer (locally advanced unresectable or metastatic) or patients progressing within 6 months of receiving neoadjuvant/adjuvant gemcitabine.

3.2.3 Use of an anti-cancer treatment drug ≤ 21 days or 5 half-lives (whichever is shorter)

prior to the first dose of Azenosertib.

3.2.4 Active use of treatment prescription or non-prescription drugs, or food and herbal supplements, that are strong/moderate CYP3A4 inhibitors, P-gp inhibitors, or strong CYP3A4 inducers.

3.2.5 Any prior palliative radiation to ≥5% of the bone marrow, and must have been completed and recovered from adverse effects of therapy at least 21 days prior to the first dose of Azenosertib.

3.2.6 Participants who have undergone major surgical procedures ≤ 28 days, or minor surgical procedures ≤ 7 days, of the first dose of Azenosertib. No waiting period is required following port-a-cath or other central venous access placement.

3.2.7 Presence of CTCAE v5.0 Grade >1 toxicity from prior therapy (except alopecia, anorexia or CTCAE grade 2 peripheral neuropathy).

3.2.8 Patient is unable to swallow oral medications. Note: Patient may not have a percutaneous endoscopic gastrostomy (PEG) tube or be receiving total parenteral nutrition (TPN).

3.2.9 Participants with known malignant central nervous system (CNS) disease other than neurologically stable, treated brain metastases – defined as metastasis having no evidence of progression or hemorrhage for at least 2 weeks after the completion of treatment (including brain radiotherapy). Must be off any systemic corticosteroids for the treatment of brain metastases for at least 14 days prior to enrollment.

3.2.10 History of hypersensitivity to compounds of similar chemical or biologic composition to gemcitabine or Azenosertib.

3.2.11 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection*, uncontrolled major seizure disorder, unstable spinal cord compression, superior vena cava syndrome, or psychiatric illness/social situations that would limit compliance with study requirements.

*Recurrent, active or suspected infection and/or patients who are predisposed to an increased risk of severe infection. Patients with infections that require antibiotics or antifungal agents may be eligible, provided that infection is resolved and treatment is completed at least 7 days prior to study treatment start.

3.2.12 Participants with a clinically significant gastrointestinal disorder that in the opinion of the treating investigator could impact the absorption of the study drugs, including but not limited to refractory nausea and vomiting, chronic gastrointestinal disease, inability to swallow the formulated product, or previous significant bowel resection that would preclude adequate absorption, distribution, metabolism, or excretion of

Azenosertib.

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

3.2.14 Administration of strong or moderate CYP3A4 inhibitors or inducers and P-gp inhibitors. (See **Appendix B**)

3.2.15 Pregnant or lactating women are excluded from this study because gemcitabine/Azenosertib are anti-cancer agents with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with gemcitabine/Azenosertib, breastfeeding should be discontinued if the mother is treated with gemcitabine/Azenosertib.

3.2.16 Any of the following cardiac diseases currently or within 6 months of the first dose of Azenosertib:

- 12-lead ECG demonstrating a corrected QT interval using Fridericia's formula (QTcF) of >470 ms, except for subjects with atrioventricular pacemakers or other conditions (e.g., right bundle branch block) that render the QT measurement invalid.

3.3 Inclusion of Women and Minorities

Both men and women of all races and ethnic groups are eligible for this trial.

4. REGISTRATION PROCEDURES

4.1 General Guidelines for DF/HCC Institutions

Institutions will register eligible participants in the Clinical Trials Management System (CTMS) OnCore. Registrations must occur prior to the initiation of any protocol-specific therapy or intervention. Any participant not registered to the protocol before protocol-specific therapy or intervention begins will be considered ineligible and registration will be denied.

An investigator will confirm eligibility criteria and a member of the study team will complete the protocol-specific eligibility checklist.

Following registration, participants may begin protocol-specific therapy and/or intervention. Issues that would cause treatment delays should be discussed with the DF/HCC Sponsor-Investigator. If the subject does not receive protocol therapy following registration, the subject must be taken off study in the CTMS (OnCore) with an appropriate date and reason entered.

4.2 Registration Process for DF/HCC Institutions

Applicable DF/HCC policy (REGIST-101) must be followed.

4.3 General Guidelines for Other Investigative Sites

Eligible participants will be entered on study centrally at the Dana-Farber Cancer Institute (DFCI) by the Study Coordinator or Project Manager. The required forms can be found in **Section 4.4**.

Following registration, participants should begin protocol therapy within 7 days. Issues that would cause treatment delays should be discussed with the sponsor-investigator. If the subject does not receive protocol therapy following registration, the subject must be taken off study in the CTMS (OnCore) with an appropriate date and reason entered.

4.4 Registration Process for Other Investigative Sites

To register a participant, the following documents should be completed by the participating site and faxed or e-mailed to the Study Coordinator/Project Manager:

- Signed participant consent form
- HIPAA authorization form
- Eligibility checklist
- Screening provider note including the medical/surgical history, ECOG performance status, vital signs, and physical exam findings
- Pathology report confirming adenocarcinoma of the pancreas
- Laboratory reports including:
    - CBC with differential
    - Chemistry panel
    - Pregnancy test (if applicable)
- Screening imaging report
- Screening EKG

The Study Coordinator or Project Manager will follow DF/HCC policy (REGIST-101) and register the participant on the protocol. The Study Coordinator or Project Manager will fax or e-mail the participant study number to the participating site.

NOTE: Registration will be conducted during the regular business hours of 8:30 AM to 4:30 PM Eastern Standard Time Monday through Friday, holidays excluded. Same day treatment registrations or registrations occurring outside of these timeframes will only be accepted with prior notice and discussion with the DF/HCC Project Manager or Study Coordinator.

5. TREATMENT PLAN

A treatment cycle will be defined as 21 consecutive days and treatment will be administered on an outpatient basis. Reported adverse events and potential risks are described in **Section 7**. Appropriate dose modifications are described in **Section 6**.

5.1 Dosing Regimen

All participants enrolled will begin treatment with gemcitabine and Azenosertib following the schedule depicted in the table below. Given the RP2D determined by the initial phase 1 cohort, DL1 is defined as IV infusion of gemcitabine 800 mg/m^2 on day 1 and 8 of each 21-day cycle. Patients will also receive Azenosertib 150 mg by mouth daily on days 1-5, 8-12, and 15-19 of every cycle. If deemed too toxic in the safety run-in, DL-1 will be used (defined as IV infusion of gemcitabine 600 mg/m^2 on day 1 and 8 of each 21-day cycle. Patients will also receive Azenosertib 150 mg by mouth daily on days 1-5, 8-12, and 15-19 of every 21-day cycle. Stages 1 and 2 of the phase 2 analysis will utilize the confirmed recommended phase 2 dose as determined by the safety run-in.

5.1.1 Safety Run-In

Following a 3+3 algorithm, an initial 3 patients will be enrolled at DL1. If 0 or 1 of 3 patients experience a DLT, an additional 3 patients will be enrolled to DL1. DL1 will be declared the confirmed RP2D (RP2D$_c$) if at most 1 of 6 patients treated at DL1 experience a DLT. Alternatively, if there are 2 or more DLTs observed at DL1, 3 patients will be enrolled at DL -1 and the 3+3 rules repeated for safety evaluation. The RP2D$_c$ will be the highest DL at which at most 1 patient of 6 experiences a DLT. If DL -1 is considered too toxic, the combination will be considered unsafe for further development at the explored dose level. Refer to **Section 13.**

Table 1: Dosing Plan

Study Agent	Assigned Dose[1,2]	Schedule	Route of Administration	Cycle length
Gemcitabine[3,4]	800 mg/m^2 (DL1)	Day 1 and 8	Intravenous	21 days
Azenosertib[3, 4]	150 mg	Days 1-5, 8-12, and 15-19	Oral	21 days

1. Body surface area (BSA) to be calculated per institutional standards of practice.
2. The gemcitabine dose will be individually 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.
3. Premedications per institutional policy and Section 5.4
4. DL-1 dose will be gemcitabine 600 mg/ m^2 on days 1 and 8 and Azenosertib at 150 mg once daily on 1-5, 8-12, and 15-19.

The participant will be requested to maintain a medication diary of each dose of Azenosertib. The medication diary will be returned to clinic staff at the end of each cycle. Participants enrolled to the safety run-in portion of the trial will be required to have received at least 75

percent of their assigned doses of Azenosertib during the first cycle to be considered evaluable for DLT purposes. Participants will be replaced if they do not meet these parameters during cycle 1 for reasons other than toxicity (e.g. withdrawal of consent for participation, or rapid disease progression and subsequent removal from the trial).

5.1.2 Simon's Two-Stage Enrollment

Once the $RP2D_c$ has been determined, we will investigate the efficacy of therapy and will include patients treated with the RP2D in the phase 2 analysis. In stage 1, 23 patients will be enrolled; all patients who were treated in the safety lead-in will be included in stage 1. If 6-month PFS rate is ≤ 21.7% (≤ 5/23 patients with at least 6-month PFS), enrollment will end, and the combination will be declared futile. If 6 or more patients have ≥ 6-month PFS, then an additional 11 patients will be enrolled for a total sample size of 34.

5.2 Pre-Treatment Criteria

5.2.1 Cycle 1, Day 1

If screening laboratory assessments were completed ≤ 5 days prior to cycle 1 day 1, laboratory tests do not need to be repeated on cycle 1 day 1 and the screening laboratory values can be used as the cycle 1 day 1 values. If cycle 1 day 1 laboratories are performed, the values do not need to re-meet eligibility criteria, participants will be allowed to initiate treatment at the treating investigator's discretion.

5.2.2 Subsequent Cycles

Management guidelines for toxicities associated with study treatment are located in **Section 6**.

5.3 Agent Administration

Table 2: Regimen Description

Agent	Premedications / Precautions	Route	Schedule	Cycle Length
Gemcitabine[1]	Per institutional practice and the FDA package insert	IV over 30 minutes[2]	per $RP2D_c$	per $RP2D_c$
Azenosertib[1]	Subjects should take Azenosertib with a meal; administration with a meal may improve tolerability and reduce gastrointestinal symptoms. Azenosertib tablets should be swallowed whole (do not chew or crush prior to swallowing). Tablets should not be ingested if they are broken, cracked, or otherwise not intact.	Oral	per $RP2D_c$	

Table 2: Regimen Description				
Agent	**Premedications / Precautions**	**Route**	**Schedule**	**Cycle Length**
1. All participants should be monitored carefully for any signs of infection (including, but not limited to, temperature ≥99.5°F/37.5°C, unexplained tachycardia, increased sweating, chills, cough, urinary symptoms suggestive of a urinary tract infection, diarrhea, or unusual abdominal pain) even in the absence of neutropenia and to perform an appropriate fever work up (including but not limited to CBC with differential, complete metabolic panel, urinalysis and culture, and other tests such as chest x-ray per symptomatology). In the event an infection is suspected, treatment with empiric antibiotics should be administered while waiting for culture results to be returned.				
2. Study sites may follow standard of care infusion time windows				

5.3.1 Gemcitabine Administration

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)
- Follow dosing administration practices per Tables 1 and 2
- Body surface area to be calculated per institutional standards of practice
- The gemcitabine dose will be individually calculated for all infusion visits according to the subject's current weight. In calculating the dose, there will be no downward adjustment to "ideal" body weight unless institutional policy requires it.

5.3.2 Azenosertib Administration

Instructions for the administration of Azenosertib:

Azenosertib is available in 25 mg and 100 mg tablets. Subjects should take Azenosertib by mouth at approximately 24-hour intervals at approximately the same time each day with food. Azenosertib tablets should be swallowed whole (do not chew or crush prior to swallowing). Tablets should not be ingested if they are broken, cracked, or otherwise not intact.

If the subject misses a dose of Azenosertib within 8 hours of the time it is usually taken, the subject should take the missed dose as soon as possible and resume the normal daily dosing schedule. If a subject misses a dose by more than 8 hours, the subject should not take the missed dose and should resume the usual dosing schedule the next day.

If the subject vomits following dosing, no additional dose should be taken that day. The next prescribed dose should be taken at the usual time.

Lab values should be reviewed and approved by the Investigator or a qualified designee prior to Azenosertib dosing at each study visit in which labs are collected. Once labs are approved, a member of the study team should contact the subject to confirm the scheduled dose of

Azenosertib should be taken.

5.4 General Concomitant Medication and Supportive Care Guidelines

No investigational or commercial agents or therapies other than Azenosertib or gemcitabine may be administered with the intent to treat the participant's malignancy. Bisphosphonate use is permitted.

Investigators should use appropriate supportive medications to address toxicities that arise during the study, including but not limited to anti-emetics, anti-diarrheals, and blood product transfusion.

5.4.1 Permitted Concomitant Medications

Permitted concomitant medications are described in the table below.

Table 3: Permitted Concomitant Medications	
Supportive Medication/Class of drug	**Usage**

Anti-emetics (e.g., NK-1 receptor antagonists, 5-hydroxytryptamine-3 antagonist, steroids, etc.) use is required. If there are no institutional standards, refer to the ASCO guidelines for anti-emetics in oncology.	In order to maximize the gastrointestinal tolerability of Azenosertib, antiemetic prophylaxis is mandatory for the first two cycles while subjects are taking Azenosertib (Note: these measures do not need to be utilized during a dosing interruption or on days in which Azenosertib is not taken for subjects on a 5 day on / 2 day off schedule). For Cycle 3 and onwards the same regimen as used in Cycle 1 and Cycle 2 may be used as needed per Investigator's discretion and following relevant guidelines and/or institutional standards for prophylactic antiemetics for chemotherapy administration. Guidelines for mandatory prophylaxis regimens are shown in **Table 12**. If the guidelines in this table cannot be followed for any reason, then discuss with PI. Premedication with a 3-drug regimen of anti-emetics is mandatory including NK1 antagonist, 5HT3 antagonist, and oral steroid. Dopamine/serotonin antagonist is preferred for breakthrough nausea and vomiting. NK1 Antagonist: Option: A: Aprepitant (125 mg) administered orally on Day 1 of each chemotherapy administration followed by 80 mg on Day 2 and Day 3, given one hour prior to Azenosertib administration. Option B: Rolapitant (180 mg) administered orally on Cycle 1 Day 1 and every two weeks thereafter, given 2 hours prior to Azenosertib administration. 5HT3-Antagonist: Option A: Ondansetron (8 mg) administered orally daily, given 30 minutes prior to Azenosertib administration. Option B: Granisetron (1 to 2 mg) administered orally daily, given one hour prior to Azenosertib administration. Oral steroid: Dexamethasone taper (8 mg on day 1 and day

	8. Followed by 4 mg, 4 mg, 2 mg, and 2 mg on days 2-5 and days 9-12. For days 15-19, administer 4 mg, 4 mg, 4 mg, 2 mg, 2 mg, respectively. Dosing can be adjusted at Investigators' discretion after cycle 1.) Dopamine/serotonin antagonist: Olanzapine (5 to 10 mg) added daily, administered orally at bedtime. Other antiemetic medications may be administered per ASCO and/or NCCN guidelines until resolved or at the Investigators' discretion.
Loperamide (Imodium)	Loperamide (Imodium) is required at the first onset of diarrhea according to ASCO guidelines and institutional standard. Please refer to **Table 11** in **section 6.3** for details.
Medications including but not limited to the following: • Bisphosphonates and receptor activator of nuclear factor kappa-B ligand (RANKL) inhibitors (eg, denosumab). • Participants requiring therapeutic warfarin or coumadin-derivative anticoagulants will be monitored with INR and Prothrombin Time (PT) as clinically indicated. • Low molecular weight heparin, rivaroxaban, or equivalent anticoagulant therapy is permitted where clinically indicated.	Medications may be administered for maintenance of existing conditions prior to study enrollment or for a new condition that develops while on study.
ASCO, American Society of Clinical Oncology; INR, International Normalized Ratio	

All medications and therapies, both prescription and over the counter (including vitamins and supplements), administered during the study will be reviewed on an ongoing basis and must be recorded in the source documents. They must be recorded in the eCRF using generic drug names when possible.

Pre-study medications and therapies administered up to 30 days prior to Cycle 1 Day 1 and medications/therapies administered on study up to 30 days after the last study drug administration (or start of subsequent cancer treatment) will be recorded.

All transfusions administered within 14 days prior to Cycle 1 Day 1 and transfusions and/or

blood product-related procedures administered on study up to 30 days after the last study drug administration will be recorded. Any surgical and medical procedures related to the disease as well as radiotherapy will also be recorded from Cycle 1 Day 1 up to 30 days after the last study drug.

5.4.2 Palliative radiotherapy

Extended field or palliative radiotherapy during the trial are prohibited.

5.4.3 Granulocyte Colony-Stimulating Factor (G-CSF) and Thrombopoietin agonist

Hematologic support may be administered as medically indicated according to the institutional site standards. If there are no standard procedures for the use of growth factors, follow the American Society of Clinical Oncology (ASCO) Guidelines for Use of Hematopoietic Colony-Stimulating Factors (Smith et al 2015).

5.4.4 Azenosertib Contraception Requirements

Women of childbearing potential (WoCBP) may be included only if acceptable contraception is in place for two weeks before study entry, for the duration of the treatment with the study drug and for 5x half-lives of Azenosertib + 6 months after the last dose of Azenosertib. Sexually active female subjects of childbearing potential must agree to use protocol-recommended method of contraception from the start of the screening period until 6 months after the last dose of study drug Azenosertib.

Male patients who are involved in the study must agree to avoid procreative and unprotected sex (i.e. using acceptable forms of contraception) and must not donate sperm during the study and for 3 months after the last dose of Azenosertib. Where the female partner is pregnant or not using effective birth control, men should be advised to abstain while in the study and for 5x half-lives of Azenosertib + 3 months after the last dose of Azenosertib. In addition, patients can not undergo egg donation/egg harvesting for 6 months after the last dose of the study drugs.

Male patients should be advised to arrange for the freezing of sperm samples prior to the start of the study should they wish to father children while on Azenosertib or during the 5x half-lives of Azenosertib + 3 months after stopping Azenosertib.

5.4.5 Prohibited Concomitant Medications

Drug interaction studies with Azenosertib have not been conducted in humans. The following information is based on results from in vitro studies. Investigators should exercise normal caution when prescribing concomitant medications with Azenosertib and are encouraged to contact the Sponsor's Medical Monitor or designee when they are unsure whether a drug should be prescribed to a subject in the clinical study.

Azenosertib is a substrate for CYP3A4 and P-gp. Therefore, strong and moderate CYP3A4 inhibitors and inducers as well as P-gp inhibitors can significantly affect plasma concentrations

of Azenosertib. (See **APPENDIX B**)

Azenosertib is a potent inhibitor of multidrug and toxin extrusion 1 (MATE1) and MATE2K, so subjects who are taking substrates of MATE1 and MATE2K should be carefully monitored for AEs that may be related to drug-drug interactions.

Azenosertib is not a potent inhibitor of other transporters and CYPs. It is however recommended to closely monitor subjects who are taking substrates of CYP3A4, P-gp, and BCRP.

Azenosertib is an inducer of CYP2B6 so subjects who are taking CYP2B6 substrates will also need to be monitored closely.

5.5 Criteria for Taking a Participant Off Protocol Therapy

Duration of therapy will depend on individual response, evidence of disease progression and tolerance. In the absence of treatment delays due to adverse event(s), treatment may continue until one of the following criteria applies:

- Disease progression (radiographic progression based on RECIST 1.1. or clinical progression in the judgement of the treating investigator)
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s)
- Participant demonstrates an inability or unwillingness to comply with the oral medication regimen and/or documentation requirements
- Participant decides to withdraw from the protocol therapy
- General or specific changes in the participant's condition render the participant unacceptable for further treatment in the judgment of the treating investigator
- Pregnancy

Participants will be removed from the protocol therapy when any of these criteria apply. The reason for removal from protocol therapy, and the date the participant was removed, must be documented in the case report form (CRF). Alternative care options will be discussed with the participant.

When a participant is removed from protocol therapy and/or is off of the study, the participant's status must be updated in OnCore in accordance with REGIST-OP-1.

5.6 Duration of Follow Up

Participants will be followed for 30 days after removal from protocol therapy or until death for all adverse events, whichever occurs first. Participants removed from protocol therapy for unacceptable adverse event(s) will be followed until resolution or stabilization of the adverse event. After completion of the 30 day follow up period, participants will continue to be followed until death after removal from protocol therapy for survival status. Participants removed from protocol therapy prior to disease progression will continue to be followed for disease response as well. No imaging or study visits will be mandated as part of this follow up, but disease response will be captured by review of any available imaging, medical records, and/or healthcare provider exams performed as part of standard of care.

5.7 Criteria for Taking a Participant Off Study

Participants will be removed from study when any of the following criteria apply:

- Lost to follow-up
- Withdrawal of consent for data submission
- Death

The reason for taking a participant off study, and the date the participant was removed, must be documented in the case report form (CRF). In addition, the study team will ensure the participant's status is updated in OnCore in accordance with REGIST-OP-1.

6. TOXICITY MANAGEMENT

Dose delays and modifications will be made as indicated in the following table(s). The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 5.0 will be utilized for dose delays and dose modifications. A copy of the CTCAE version 5.0 can be downloaded from the CTEP website http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

6.1 Dose Delays

The study agents may be held for a maximum of 21 days to allow for recovery of toxicity. Participants requiring a longer delay should be removed from protocol therapy. Participants who are deriving clinical benefit who require a longer hold may be allowed to continue treatment following discussion and agreement with the sponsor-investigator.

If attribution of the toxicity is known, study medications may be held independently of each other. For example, a participant may continue dosing with Azenosertib while holding gemcitabine at the treating investigator's discretion. If attribution of the toxicity is unknown or the combination of study agents may be contributing to the toxicity, both study agents must be held.

In the event of a dose hold due to toxicity, the counting of cycle days and protocol assessment

schedule will continue without interruption. For example, a participant who holds dosing of Azenosertib on Cycle 2 Day 17 for toxicity will resume dosing with Cycle 3 and proceed with their next scheduled clinic visit as previously planned (Cycle 3 Day 1). Interim visits may be conducted as clinically necessary to manage toxicity; however, the cycle will not restart and doses will not be "made up" in the event of dosing delays due to toxicity.

6.2 Dose Modifications

If attribution of the toxicity is known, dose reductions may occur independently of each other. For example, the dose of Azenosertib may be reduced while maintaining the dose of gemcitabine. If toxicity attribution is unknown or the combination of study agents may contribute to the toxicity, both agents must be dose reduced.

If attribution of the toxicity is known, discontinuation of study medications may also occur independently of each other with agreement from the sponsor-investigator. For example, participants who cannot tolerate gemcitabine may be allowed to continue to receive Azenosertib if the sponsor-investigator agrees.

Dose reductions may be made for participants enrolled in accordance with the tables below. Once a participant's dose of gemcitabine or Azenosertib has been reduced, it may not be re-escalated. No further dose reductions will be allowed beyond the modification tables below.

Table 4: Azenosertib Dose Modification Levels		
Starting Dose Level	**Dose Reduction 1**	**Dose Reduction 2**
150 mg 5 days on/ 2 days off	100 mg 5 days on/ 2 days off	50 mg 5 days on/ 2 days off

Table 5: Gemcitabine Dose Modification Levels			
Starting Dose Level	**Dose Reduction 1**	**Dose Reduction 2**	**Dose Reduction 3**
800 mg/m^2 days 1, 8 of 21 day cycle	600 mg/m^2 days 1, 8 of 21 day cycle	400 mg/m^2 days 1, 8 of 21 day cycle	200 mg/m^2 days 1, 8 of 21 day cycle

6.3 Toxicity Management Guidelines

Management of toxicity considered ***at least possibly related*** to gemcitabine and/or Azenosertib is detailed below.

All participants should be monitored carefully for any signs of infection (including, but not limited to, temperature ≥99.5°F/37.5°C, unexplained tachycardia, increased sweating, chills, cough, urinary symptoms suggestive of a urinary tract infection, diarrhea, or unusual abdominal pain) even in the absence of neutropenia and to perform an appropriate fever work up (including but not limited to CBC with differential, complete metabolic panel, urinalysis and culture, and other tests such as chest x-ray per symptomatology). In the event an infection is suspected,

treatment with empiric antibiotics should be administered while waiting for culture results to be returned.

6.3.1 Dose Limiting Toxicity during lead-in

A Dose Limiting Toxicity (DLT) is defined as any AE (except clearly attributable to an extraneous cause, such as an underlying disease) occurring in Cycle 1 and meeting at least one of the following criteria in Table 6 below. Cycle 1 is 21 days.

Table 6: Criteria for Dose Limiting toxicity

Hematologic Toxicity	Grade 4 thrombocytopenia Grade 3 thrombocytopenia associated with bleeding, and/or requirement for transfusion and/or lasting > 7 days.
	Grade 4 neutropenia > 7 consecutive days despite possible hematologic growth factor support.
	Febrile neutropenia (ANC < 1.0×10^9/L and fever ≥ 38.3°C) or a sustained temperature >38°C for more than one hour, requiring initiation of antibiotics.
Non-Hematologic Toxicity	Any non-hematologic toxicity ≥ grade 3 considered by the investigator to be associated with the study drugs and not clearly due to the patient's cancer or extraneous causes.[a] This excludes alopecia and includes only diarrhea, nausea or vomiting when optimal prophylactic measures have been prescribed. Hepatic toxicity satisfying Hy's law defined as the combined elevations in serum ALT >3 times the upper limit of normal (ULN) and total bilirubin >2 ULN in the absence of alkaline phosphatase (ALP) elevation (<2 ULN). Grade 3+ electrolyte abnormality that lasts >72 hours, unless the patient has clinical symptoms, in which case all grade 3+ electrolyte abnormality regardless of duration should count as a DLT.
Other	Any AE leading to a Azenosertib Cycle 1 dose intensity of < 75% and/or a dose intensity of the gemcitabine of <75% (except if clearly attributable to extraneous cause. A delay in therapy of >2 weeks due to treatment-associated toxicity will be considered dose limiting. 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.

[a] For any Grade 3 or 4 hepatic toxicity not resolved within 7 days to Grade 0-1 (or Grade 2 if liver metastases), an abdominal computed tomography scan should be performed to assess if it is related to disease progression.

Participants enrolled to the safety run-in portion of the trial will be required to have received at least 75 percent of their assigned doses of Azenosertib during the first cycle to be considered evaluable for DLT purposes. Participants will be replaced if they do not meet these parameters during cycle 1 for reasons other than toxicity (e.g. withdrawal of consent for participation, or rapid disease progression and subsequent removal from the trial).

6.3.2 Hematologic Toxicity Management Guidelines

Table 7: Hematologic Toxicity Management Guidelines

Event Term	Event Grade / Description	1st Event Action	2nd Event Action	3rd Event Action
Neutrophil Count Decreased[1]	Grade 2	Investigator judgement to continue Azenosertib and gemcitabine or interrupt until grade 1.		
Neutrophil Count Decreased[1, 4]	Grade 3 **without** documented infection and/or fever	Hold gemcitabine and Azenosertib until resolution to grade 1. Investigator discretion to keep next gemcitabine dose the same. Reduce Azenosertib dose by one dose level.	Hold gemcitabine and Azenosertib until resolution to grade 1. Investigator discretion to keep next gemcitabine dose the same. Reduce Azenosertib dose by one dose level	Hold gemcitabine and Azenosertib until resolution to grade 1. Investigator discretion to keep next gemcitabine dose the same or reduce the dose by one dose level. Discontinue Azenosertib.
Neutrophil Count Decreased[1, 4]	Grade 3 < 1.0-0.5 x 10^9/L with documented infection and/or fever	Hold Azenosertib and gemcitabine until resolution to grade 1. Resume Azenosertib and gemcitabine at next reduced dose modification level	Discontinue Azenosertib and gemcitabine and follow for disease progression[6]	
Neutrophil Count Decreased[1, 4]	Grade 4 **without** documented infection and/or fever	Hold gemcitabine and Azenosertib until resolution to grade 1. Investigator discretion to keep gemcitabine dose the same or reduce the dose by one dose modification level. Reduce Azenosertib dose by one dose level	Hold gemcitabine and Azenosertib until resolution to grade 1. Resume gemcitabine and Azenosertib with one dose modification level reduction.	Resume gemcitabine. Discontinue Azenosertib.
Neutrophil Count Decreased[1]	Grade 4 Febrile neutropenia or Grade 4 infection with Grade 4 neutropenia (< 0.5 x 109/L)	Discontinue gemcitabine and Azenosertib and follow for disease progression		
Grade ≥3 infection with neutropenia[1,2, 4]	Grade ≥3	Hold until resolution. Resume gemcitabine and Azenosertib with one dose modficiation level reduction.	Resume gemcitabine and discontinue Azenosertib.	

Table 7: Hematologic Toxicity Management Guidelines				
Event Term	**Event Grade / Description**	**1st Event Action**	**2nd Event Action**	**3rd Event Action**
Platelet Count Decreased[1]	Grade 2 **without** clinically significant bleeding	Hold until resolution to grade 1. Investigator discretion to keep next Azenosertib and/or gemcitabine dose the same or reduce the dose by one dose modification level.	Hold until resolution to grade 1. Investigator discretion to keep gemcitabine dose the same or reduce the dose by one dose level. Reduce Azenosertib dose by one dose modification level.	Hold until resolution to grade 1. Investigator discretion to keep gemcitabine dose the same or reduce the dose by one dose modification level. Reduce Azenosertib dose by one dose modification level.
Platelet Count Decreased[1]	Grade 2 **with** clinically significant bleeding	Hold until resolution to grade 1. Consider transfusion per institutional guidelines. Resume gemcitabine and/or Azenosertib with one dose modification level reduction.	Hold until resolution to grade 1. Consider transfusion per institutional guidelines. Resume gemcitabine and/or Azenosertib with one dose modification level reduction.	Permanently discontinue study treatment.
Platelet Count Decreased[1]	Grade 3 **without** clinically significant bleeding	Hold until resolution to grade 1. Resume gemcitabine and/or Azenosertib with one dose modification level reduction.	Hold until resolution to grade 1. Resume gemcitabine and/or Azenosertib with one dose modification level reduction.	Discontinue gemcitabine and Azenosertib and follow for disease progression.
Platelet Count Decreased[1]	Grade 4 **without** clinically significant bleeding	Hold gemcitabine and Azenosertib until resolution to grade 1. Resume gemcitabine and Azenosertib with one dose modification level reduction.	Permanently discontinue study treatment.	NA
Platelet Count Decreased[1]	Thrombocytopenic hemorrhage (clinically significant bleeding) associated with platelet count < 50,000/ µL	Permanently discontinue study treatment.		
Anemia[3]	Grade 2	Investigator judgement to continue Azenosertib and/or gemcitabine with supportive treatment or interrupt dose. If repeat Hgb < 9, interrupt Azenosertib and/or gemcitabine for a maximum of 28 days until resolution to Hgb ≥ 9 g/dL or baseline.		
Anemia[3]	≥ Grade 3	Interrupt Azenosertib and gemcitabine until resolution to Hgb ≥ 9 g/dL. Upon recovery, consider dose reducing gemcitabine by one dose modification level. Dose reduce Azenosertib by one dose level. In case of a repeat episode of Hb <8g/dL, interrupt dose for a maximum of 21 days until Hb ≥9 g/dL, and upon recovery, further dose reduce Azenosertib. In case of a third episode of Hb <8g/dL, discontinue gemcitabine and Azenosertib.		

Table 7: Hematologic Toxicity Management Guidelines				
Event Term	**Event Grade / Description**	**1st Event Action**	**2nd Event Action**	**3rd Event Action**
1. If the patient has concurrent neutropenia and thrombocytopenia, please follow the most conservative guidance and discuss with the sponsor-investigator as needed. **If neutropenia or thrombocytopenia occurs, complete blood count (CBC) should be monitored at least weekly until resolution.** 2. Participants experiencing febrile neutropenia with significant symptoms should be managed in a hospital setting according to standard procedures, with the urgent initiation of intravenous (IV) antibiotic therapy and interruption of study treatment. 3. Give appropriate supportive treatment and investigate causality. 4. Consider administering G-CSF				

Neutropenia and/or Febrile neutropenia

Participants experiencing febrile neutropenia and patients with severe neutropenia at high risk of sepsis should be managed in a hospital setting according to standard procedures, with interruption of study treatment, the urgent empiric initiation of intravenous (IV) antibiotic therapy, and use of granulocyte colony-stimulating factor (G-CSF) according to institutional standards.

Antibiotics and G-CSF are recommended in cases of severe neutropenia, to be administered for 3 days or until the neutrophil count improves to ≤ Grade 2. G-CSF is not recommended within 24 hours of the last dose of Azenosertib unless necessary. G-CSF administration should commence 24 – 48 hours after last dose of Azenosertib. Growth factor support should be stopped at least 24 hours before the next dose of Azenosertib (7 days for pegylated G-CSF). Growth factor support may be initiated per ASCO or local institutional guidelines at the treating investigator's discretion during or following the first occurrence of neutropenic fever.

Participants with febrile neutropenia without symptoms should be managed according to standard institutional guidelines.

For patients experiencing severe neutropenia in a preceding cycle, secondary prophylaxis with (non-pegylated) G-CSF daily for 3 days commencing 24 – 48 hours after last dose of Azenosertib in a treatment cycle is recommended. Secondary prophylaxis is to be administered in addition to any required dose reduction.

Please note that G-CSF is not recommended within 24 hours of the last dose of study treatment. Non-pegylated G-CSF should be stopped at least 48 hours before restarting study drug, and pegylated G-CSF should be stopped 14 days before restarting study drug.

Renal function (measured by creatinine clearance) should be monitored and dose modifications to manage renal impairment followed as Azenosertib exposure and the risk of severe neutropenia might be increased in patients with low creatinine clearance. Further information on renal function impairment management can be found in section 6.3.5.

6.3.3 Non-Hematologic Toxicity Management Guidelines

Table 8: Management of Non-Hematological Toxicities

Grade	Action
Grade 1/Grade 2	Investigator judgement to initiate supportive treatment and continue Azenosertib, or interrupt dose for a maximum of 21 days. For persistent Grade 2 adverse events despite maximum supportive treatment, dosing may be resumed at next reduced dose/schedule modification level, as clinically indicated.
Grade 3[a] Grade 3 toxicity for ≤ 21 days Grade 3 toxicity for ≥ 21 days	Initiate supportive treatment, interrupt Azenosertib for a maximum of 21 days, until improved to ≤ Grade 1, and follow algorithm as below: • Resume at next reduced dose/schedule modification level, maintaining supportive treatment. • At recurrence, dosing may be resumed only with a further dose/schedule modification level reduction, maintaining supportive treatment. Up to a maximum of two dose reductions are permitted. • Discontinue treatment, unless approved by the Medical Monitor.[b]
Grade 4[a] Grade 4 toxicity for ≤ 21 days Grade 4 toxicity for ≥ 21 days	Initiate supportive treatment interrupt Azenosertib for a maximum of 21 days, until improved to ≤ Grade 1 and follow algorithm as below: • Resume at next reduced dose/schedule modification level, maintaining supportive treatment. • At recurrence, discontinue treatment. • Discontinue treatment

[a] This applies to clinically significant toxicities that have been treated with appropriate supportive care.
[b] In exceptional situations where it is considered that benefits outweigh the risks, the patient may continue dosing only after approval by the Principal Investigator.

6.3.4 Management of QTc interval prolongation

ECG should be assessed if electrolyte abnormalities are detected or suspected (eg, in relation to gastrointestinal toxicity). If a prolongation of the QTc interval is detected, this must be closely monitored as clinically indicated. Dose modification guidance for QTc interval prolongation is presented in the table below.

Table 9: QTc Interval Prolongation (Azenosertib Dose Modifications)	
QTc Value (triplicate)[a,b]	**Action to be Taken**
QTc 471-500 ms (Grade 2)	Hold treatment for up to 21 days. Consider seeking cardiologist's advice. Once QTc interval has returned to Grade 1 and correction of possible electrolyte imbalance has been made, treatment may resume at the same dose level. If second occurrence, discontinue treatment permanently.
QTc ≥ 501 ms or Shift from baseline of ≥ 60 ms (Grade 3)	Hold treatment for up to 21 days. Seek cardiologist's advice and correct any electrolyte imbalance. If QTc interval recovers to ≤ 480 ms (Grade 1) or baseline if there was a shift from baseline of ≥ 60 ms, then treatment may resume at next reduced dose level, if cardiologist agrees. If QTc interval doesn't recover to ≤ 480 ms or baseline if there was a shift from baseline of ≥ 60 ms, then discontinue treatment permanently. If second occurrence, discontinue treatment permanently.
Torsades de pointes or polymorphic ventricular tachycardia or signs/symptoms of serious arrhythmia (Grade 4)·	Discontinue treatment permanently. Seek cardiologist's advice.
[a] Mean triplicate value [b] Per Common Terminology Criteria for Adverse Events Version 5.0	

6.3.5 Management of Renal Function Impairment

Creatinine clearance should be monitored intracycle (estimated by the Cockcroft-Gault equation) at the timepoints per the schedule of assessments. Treatment interruptions up to a maximum of 21 days are permitted in the event of toxicity.

Patients should be managed as per table below:

Table 10: Management of Renal Impairment	
Renal Impairment	**Azenosertib Dose Adjustment**
Moderate (CrCl 31 - 50 ml/min)	Hold dosing, introduce supportive measures (including hydration) Upon recovery to >50 ml/min resume at same dose. If CrCl does not recover to > 50 ml/min, resume at next reduced dose/schedule modification level[a].

Table 10: Management of Renal Impairment	
Renal Impairment	**Azenosertib Dose Adjustment**
Severe (CrCl ≤ 30 ml/min)	Hold dosing, introduce supportive measures (including hydration) Upon recovery to >30 ml/min, resume at next reduced dose level[a]. If CrCl does not recover to >30 ml/min within 21 days or at repeat occurrence of CrCL <30 ml/min discontinue dosing
a. In the absence of a further clinically relevant decrease in CrCl, the investigator may use their judgment to determine whether a further dose modification or discontinuation of Azenosertib is necessary.	

6.3.6 Management of Gastrointestinal Toxicity

To monitor the safety and tolerability of the study drugs, each participant will be contacted for a general assessment on Day 3 (±1 day) of Cycle 1 and 2 to assess for AEs potentially related to Azenosertib. In the event of significant diarrhea, nausea and/or vomiting, or reduced oral intake, it is strongly recommended that the participant is considered for IV fluid rehydration therapy and supportive care measures.

For participants experiencing gastrointestinal toxicities or who are otherwise at risk of dehydration, creatinine clearance should be closely monitored and dose modifications to manage renal impairment followed (see previous section).

Due to frequent reports of diarrhea with Azenosertib administration, anti-diarrheal treatment with loperamide (Imodium) is required at the **first** onset of diarrhea according to American Society of Clinical Oncology (ASCO) guidelines and institutional standard.

Participants should be instructed to notify the investigator or research staff of the occurrence of bloody or black stools, symptoms of dehydration, fever, inability to take liquids by mouth, and inability to control diarrhea within 24 hours of using loperamide or other prescribed anti-diarrheal medications.

If diarrhea is severe (ie, requiring IV rehydration) and/or associated with fever or severe neutropenia (Grade 3 or 4), broad-spectrum antibiotics must be prescribed. Participants with severe diarrhea or any diarrhea associated with severe nausea or vomiting should be hospitalised for IV hydration and correction of electrolyte imbalances.

Participants should be managed as medically indicated in Table 11.

Table 11: Management of Diarrhea	
Grade/GI toxicity	**Action**

	Investigator to provide patient with prescription for loperamide to enable immediate initiation of anti-diarrheal treatment.
Grade 2 Diarrhea	• Initiate anti-diarrheal treatment immediately with oral loperamide (maximum of 16 mg/24 hours). • Maintain liberal oral fluid intake. Participants should also be counselled to start a BRAT (bananas, rice, applesauce, toast) diet. • Investigator judgement to continue Azenosertib with supportive treatment or interrupt dose for a maximum of 21 days • For persistent Grade 2 diarrhea despite maximum supportive treatment, dosing may be resumed at next reduced dose level, as clinically indicated.
Grade 3/ Grade 4[a] Diarrhea	• Initiate anti-diarrheal treatment with oral loperamide 4 mg (maximum of 16 mg/24 hours). • Consider admission for IV rehydration and correction of electrolyte imbalances. • Interrupt Azenosertib for a maximum of 21 days, until improved to ≤ Grade 1.
Grade 3 toxicity for ≤ 21 days	• Upon recovery, resume at next reduced dose modification level, maintaining supportive treatment. • For recurrent Grade 3, dosing may be resumed only with a further dose level reduction, maintaining supportive treatment. Up to a maximum of two dose reductions are permitted.
Grade 3 toxicity for ≥ 21 days	• Discontinue treatment, unless approved by the Medical Monitor.[b]
Grade 4 toxicity for ≤ 21 days	• Upon recovery, resume at next reduced dose level, maintaining supportive treatment. • For recurrent Grade 4, discontinue treatment.
Grade 4 toxicity for ≥ 21 days	• Discontinue treatment.
GI, gastrointestinal; IV, intravenous [a] Despite maximal supportive care. [b] In exceptional situations where it is considered that benefits outweigh the risks, the patient may continue dosing only after approval by the Medical Monitor.	

Nausea and vomiting

To maximize the gastrointestinal tolerability of Azenosertib, antiemetic prophylaxis is mandatory for the first two cycles while subjects are taking Azenosertib (Note: these measures do not need to be utilized during a dosing interruption or on days in which Azenosertib is not taken for subjects on a 5 day on / 2 day off schedule or other intermittent schedules). The same regimen may be used from Cycle 3 onwards, as needed per Investigator's discretion. Guidelines for mandatory prophylaxis regimens are shown in the table below. If the guidelines in this table cannot be followed for any reason, the Investigator must consult with the Sponsor before initiating treatment.

Participants should be managed as medically indicated in Table 12.

Table 12: Prophylaxis for Nausea and Vomiting	
Antiemetic Class	Subjects ≥18 Years of Age
NK1 Receptor Antagonists	Aprepitant (125 mg) administered orally on the first day of each chemotherapy administration, followed by 80 mg on days 2 and 3, given one hour prior to Azenosertib administration OR Rolapitant (180 mg) administered orally on Cycle 1 Day 1 and every two weeks thereafter, given two hours prior Azenosertib administration AND
5-HT3 Receptor Antagonists	Ondansetron (8 mg) administered orally daily, given 30 minutes prior to Azenosertib administration[a] OR Granisetron (1 to 2 mg) administered orally daily, given one hour prior to Azenosertib administration[a] AND
Oral Steroid	Dexamethasone (8 mg on day 1 and day 8. Followed by 4 mg, 4 mg, 2 mg, and 2 mg on days 2-5 and days 9-12. For days 15-19, administer 4 mg, 4 mg, 4 mg, 2 mg, 2 mg, respectively. Dosing can be adjusted at Investigators' discretion after cycle 1.)
For Breakthrough Nausea and/or Vomiting	
Dopamine/ serotonin Antagonist	Preferred option: olanzapine (5 to 10 mg) added daily to Options A or B, administered orally at bedtime. Other antiemetic medications may be administered per ASCO and/or NCCN guidelines[a,b] until resolved, or continued at the Investigator's discretion
Abbreviations: 5-HT3, 5-hydroxytryptamine (serotonin receptor antagonists); ASCO, American Society of Clinical Oncology; NCCN, National Comprehensive Cancer Network; NK1, neurokinin 1 Note: This prophylaxis antiemetic regimen is mandatory for the first two cycles while subjects are taking Azenosertib. If recommendations cannot be followed for any reason, the Investigator must consult with the Sponsor. a Adapted from ASCO guidelines[36] b Adapted from NCCN guidelines[37]	

Additionally, consider the following guidance:

- Azenosertib should be taken with a meal; administration with a meal may improve tolerability and reduce gastrointestinal symptoms.
- Azenosertib may be administered at night instead of morning.
- Recommended starting doses of olanzapine are lower for women and elderly patients.
- Females of reproductive potential using hormonal contraceptives should use an effective alternative or back-up non-hormonal contraceptive (such as condoms and spermicides)

during treatment with aprepitant and for 1 month following the last dose of aprepitant. See Section 5.4.4 for additional guidance on contraception.

- Please follow the locally approved prescribing information or SmPC for each medication and counsel subjects on the potential side effects.
- Encourage subjects to eat small meals and snack frequently (of food that appeals to them), avoid mixing very hot or very cold food and drink together, and try to eat foods that are gentle on the stomach, possibly giving examples such as bananas, rice, applesauce, and toast. Subjects should not miss snacks or meals as nausea can worsen with an empty stomach.

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

6.4 Overdose

There is no known antidote to gemcitabine or Azenosertib. Any patient who inadvertently receives a higher dose of gemcitabine or Azenosertib than stated in the protocol should be monitored, and if symptomatic, treated with appropriate supportive care until recovery. Such occurrences, any effects, and any follow up should be reported as per the reporting guidelines in **Section 7**.

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of reported and/or potential AEs (**Section 7.1**) and the characteristics of an observed AE (**Section 7.2, Section 7.3, and Section 7.4**) will determine whether the event requires expedited reporting **in addition** to routine reporting.

7.1 Expected Toxicities

7.1.1 Expected Toxicities for Azenosertib

Refer to the most recent Azenosertib IB for detailed information. Any fatal or life-threatening events (i.e. events posing an immediate risk of dying) will always be considered unexpected for the purposes of suspected unexpected serious adverse reaction (SUSAR) reporting.

7.1.2 Expected Toxicities for Gemcitabine

The table below summarizes treatment-emergent adverse events considered expected for gemcitabine serious adverse reaction reporting. Refer to the FDA package insert for detailed information.

Table 13: Expected Toxicities for Gemcitabine	
System Organ Class	**Preferred Term**
Gastrointestinal Disorders	Nausea
	Vomiting
	Diarrhea
	Stomatitis
Investigations	Aspartate aminotransferase increased
	Alanine aminotransferase increased
	Alkaline phosphatase increased
	Blood bilirubin increased
	Creatinine increased
	Neutrophil count decreased
	Platelet count decreased
Infection and Infestations	Infection
	Sepsis
Injury, Poisoning and Procedural Complications	Infusion related reaction
Blood and Lymphatic System Disorders	Anemia
	Febrile Neutropenia
Renal and Urinary Disorders	Proteinuria
	Hematuria
General Disorders and Administration Site Conditions	Fever
	Peripheral edema
	Flu-like symptoms
Skin and Subcutaneous Tissue Disorders	Rash
	Alopecia
Respiratory, Thoracic and Mediastinal Disorders	Dyspnea
Nervous System Disorders	Somnolence
	Paresthesias

7.2 Adverse Event Definition

An AE is any untoward medical occurrence in a clinical study subject administered a medicinal product, which does not necessarily have a causal relationship with the treatment. An AE can therefore be any unfavorable and/or unintended sign, symptom, or disease temporally associated with the use of a medicinal product, whether or not considered related to the medicinal product. AEs may also include pre- or post-treatment complications that occur as a result of protocol-specified procedures, lack of clinical activity, overdose, drug abuse/misuse reports, or occupational exposure. Pre-existing events that increase in severity or change in nature during or as a consequence of participation in the clinical study will also be considered AEs.

An AE does not include the following:

- Medical or surgical procedures such as surgery, endoscopy, tooth extraction, and transfusion. The condition that led to the procedure may be an AE and must be reported.

- Pre-existing diseases, conditions, or laboratory abnormalities present or detected before the Screening Visit that do not worsen.
- Situations where an untoward medical occurrence has not occurred (e.g., hospitalization for elective surgery, social and/or convenience admissions).
- The disease/disorder being studied or expected progression, signs, or symptoms of the disease/disorder being studied.
- Anticipated day-to-day fluctuations of pre-existing disease(s) or condition(s) present or detected at the start of the study that do not worsen.
- Overdose without clinical sequelae.
- Any medical condition or clinically significant laboratory abnormality findings or other abnormal safety assessments (with an onset date before Cycle 1 Day 1 and not related to a protocol-associated procedure), which are associated with the underlying disease, unless judged by the Investigator to be more severe than expected for the subject's condition is not an AE. It is considered to be pre-existing and should be documented on the Medical History eCRF.

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

- **For expedited reporting purposes only:**
  - AEs for the agent(s) that are listed above should be reported only if the adverse event varies in nature, intensity or frequency from the expected toxicity information which is provided.
  - Other AEs for the protocol that do not require expedited reporting are outlined in the **Section 7.5** (Expedited Adverse Event Reporting) under the sub-heading of Protocol-Specific Expedited Adverse Event Reporting Exclusions.

- **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.
  - Unrelated – The AE *is clearly NOT related* to the study treatment.

7.3 Serious Adverse Events

7.3.1 Definition of Serious Adverse Events (SAE)

An SAE or serious adverse drug reaction is defined as an event that, at any dose, results in the following:

- Death.
- Life-threatening (Note: The term "life-threatening" in the definition of "serious" refers to an event in which the subject was at risk of death at the time of the event; it does not refer to an event that hypothetically might have caused death if it were more severe.).
- In-patient hospitalization or prolongation of existing hospitalization.
- Persistent or significant disability/incapacity.
- A congenital anomaly/birth defect.
- A medically important event or reaction: such events may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes constituting SAEs. Medical and scientific judgment must be exercised to determine whether such an event is reportable under expedited reporting rules. Examples of medically important events include intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; and development of drug dependency or drug abuse. For the avoidance of doubt, infections resulting from contaminated medicinal product will be considered a medically important event and subject to expedited reporting requirements.

Clarification of Serious Adverse Events:

- Death is an outcome of an AE, and not an AE in itself.
- An SAE may occur even if the subject was not on study drug at the time of occurrence of the event. Dosing may have been given as treatment cycles or interrupted temporarily before the onset of the SAE.
- "Life-threatening" means that the subject was at immediate risk of death from the event as it occurred. This does not include an event that might have led to death if it had occurred with greater severity.
- Complications that occur during hospitalizations are AEs. If a complication prolongs the hospitalization, it is a SAE.
- "In-patient hospitalization" means the subject is formally admitted to a hospital for medical reasons, for any length of time. This may or may not be overnight. It does not include presentation and care within an emergency department.
- The Investigator should attempt to establish a diagnosis of the event on the basis of signs, symptoms and/or other clinical information. In such cases, the diagnosis should be documented as the AE and/or SAE and not the individual signs/symptoms.

Given the endpoints of the study, in order to maintain the integrity of the study, the following events that are assessed as unrelated to study drug will not be considered SAEs:

- Progression of underlying disease (cancer)
- Death related to progression of underlying disease (cancer)

The Investigator or qualified sub-Investigator is responsible for assessing AEs and SAEs for causality and severity, and for final review and confirmation of accuracy of event information and assessments.

7.4 Adverse Events of Special Interest

Adverse events of special interest (AESI) for azenosertib are the following:

- Febrile neutropenia
- Neutropenia
- Neutropenic shock
- Neutrophil count decreased
- Neutrophil percent decreased
- Pancytopenia or platelet count decreased
- Thrombocytopenia

Any grade 3 or higher lab value must be recorded as an adverse event of special interest (AESI). AEs should not be recorded as AESIs unless associated with clinical signs or symptoms, requires an intervention, results in an SAE, or results in study termination or interruptions/discontinuation of study treatment for grade 1-2 AESIs. AESIs should follow the same reporting criteria as SAEs.

7.5 Adverse Event Reporting

7.5.1 In the event of an unanticipated problem or life-threatening complications treating investigators must immediately (i.e. within 24 hours of awareness) notify the DF/HCC sponsor-investigator.

7.5.2 Investigators **must** report to the DF/HCC sponsor-investigator any adverse event (AE) that occurs after the initial dose of study treatment, during treatment, or within 30 days of the last dose of treatment on the local institutional SAE form.

7.5.3 DF/HCC Adverse Event Reporting Guidelines

Investigative sites within DF/HCC will report AEs directly to the DFCI Office for Human Research Studies (OHRS) per the DFCI IRB reporting policy.

Other investigative sites will report AEs to their respective IRB according to the local IRB's policies and procedures in reporting adverse events. A copy of the submitted institutional AE form should be forwarded to the DF/HCC sponsor-investigator within the timeframes detailed in the table below.

Attribution	DF/HCC Reportable Adverse Events(AEs)				
	Gr. 2 & 3 AE Expected	**Gr. 2 & 3 AE Unexpected**	**Gr. 4 AE Expected**	**Gr. 4 AE Unexpected**	**Gr. 5 AE Expected or Unexpected**
Unrelated Unlikely	Not required	Not required	5 calendar days#	5 calendar days	24 hours*
Possible Probable Definite	Not required	5 calendar days	5 calendar days#	5 calendar days	24 hours*
# If listed in protocol as expected and not requiring expedited reporting, event does not need to be reported.					
* For participants enrolled and actively participating in the study ***or*** for AEs occurring within 30 days of the last intervention, events must be reported within 1 business day of learning of the event.					

The DF/HCC sponsor-investigator will submit AE reports from outside institutions to the DFCI OHRS according to DFCI IRB policies and procedures in reporting adverse events.

7.6 Reporting to Zentalis

The Sponsor shall forward all valid initial SAE reports and medically significant follow-up for all SAEs including non-serious Pregnancy and Breast-feeding Exposures, and events of Overdose for the involved Manufacturer's Study Drug(s) to the Manufacturer in form and format specified below to ZentalisPV@primevigilance.com within the following timelines.

Type of Cases	**Exchange Timelines**	**Format**	**Mode of Exchange**
Death/Life-Threatening (D/LT) SAEs regardless of causal relationship to Study Drug	Within **four (4) calendar days** of Sponsor's Awareness Date	MedWatch or CIOMS-I	Secure eMail (or Fax)
Other SAEs regardless of causal relationship to Study Drug, and all events of Overdose, Pregnancy and Breast-feeding exposures	Within **eight (8) calendar days** of Sponsor's Awareness Date	MedWatch or CIOMS-I	Secure eMail (or Fax)

• SAEs for which Subject did not receive Manufacturer's Study Drug should also be forwarded to Manufacturer. This includes, but is not limited to, SAEs which occur after informed consent is signed and prior to first dose of study drug(s), or where the subject has received another protocol-specified study drug, but has not received azenosertib.
• Sponsor shall be responsible for obtaining follow-up information on the reports from the Study.
• Non-serious Adverse Events will be forwarded by means of a study update report at the end of each quarter of the Study, and by means of a study report at the end of the study.

7.7 Reporting to the Food and Drug Administration (FDA)

The DF/HCC sponsor-investigator, as study sponsor, will be responsible for all communications with the FDA. The sponsor-investigator will report to the FDA, regardless of the site of occurrence, any serious adverse event that meets the FDA's criteria for expedited reporting following the reporting requirements and timelines set by the FDA.

7.8 Reporting to Hospital Risk Management

Participating investigators will report to their local Risk Management office any participant safety reports, sentinel events or unanticipated problems that require reporting per institutional policy.

7.9 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions to the DF/HCC sponsor-investigator on the toxicity case report forms. **AEs reported through expedited processes (e.g., reported to the IRB, FDA, etc.) must <u>also</u> be reported in routine study data submissions.**

8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational agents administered in this study can be found in **Section 7.1**.

8.1 Azenosertib

Azenosertib, also known as KP-2638 (both Azenosertib and KP-2638 are used interchangeably), is a novel, selective, and orally bioavailable small-molecular inhibitor of the WEE1 tyrosine kinase.

8.1.1 Description

Molecular Formula: $C_{29}H_{34}N_8O_2$

Relative Molecular Mass: molecular weight of 526.63 g/mol

8.1.2 Form

Azenosertib initial formulation is supplied as film-coated tablets containing 25 mg or 100 mg of Azenosertib as an active ingredient for oral administration. Azenosertib registrational formulation drug product is supplied as 50-mg and 100-mg film-coated tablets. The formulation

is composed of azenosertib drug substance, microcrystalline cellulose, lactose monohydrate, polyvinylpyrrolidone, croscarmellose sodium, sodium stearyl, fumarate, colloidal silicon dioxide, and magnesium stearate.

Refer to the Investigator's Brochure for additional details on the formulation, appearance, packaging, and labeling of Azenosertib.

8.1.3 Storage and Stability

Until dispensed to the subjects, all study drug should be stored in a securely locked area, accessible only to authorized site personnel. To ensure the stability and proper identification, the drug product should not be stored in a container other than the container in which it was supplied. Product storage instructions are briefly described below for Azenosertib and the combination products. Refer to the Investigator's Brochure for further details.

Azenosertib will be supplied (and labeled per local regulation) as tablets containing 25 mg, 50 mg, or 100 mg of Azenosertib and stored at controlled room temperature (20-25°C), protected from light, with excursions allowed to 15-30°C until needed for administration.

8.1.4 Handling

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of the chemotherapeutic agent in a self-contained and protective environment. It is acceptable to remove and destroy excess pills if needed per institute standard, while dispensing the needed quantity in original container

8.1.5 Availability

Azenosertib is an investigational agent that will be supplied by Zentalis Pharmaceuticals.

8.1.6 Administration

Refer to **Section 5**.

8.1.7 Ordering

Azenosertib will be ordered by site pharmacy personnel from Zentalis Pharmaceuticals.

8.1.8 Accountability

The investigator, or a responsible party designated by the investigator, should maintain a careful record of the inventory and disposition of the agent using the NCI Drug Accountability Record Form (DARF) or another comparable drug accountability form. (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.)

8.1.9 Destruction and Return

Expired, returned, or unused supplies of Azenosertib should be destroyed according to institutional policies. Destruction will be documented in the Drug Accountability Record Form.

8.2 Gemcitabine

8.2.1 Description

Refer to the FDA package insert. Gemcitabine is a sterile white to off-white powder. The empirical formula for gemcitabine hydrochloride is $C_9H_{11}F_2N_3O_4$ • HCl. It has a molecular weight of 299.66 g/mol. Gemcitabine hydrochloride is soluble in water, slightly soluble in methanol, and practically insoluble in ethanol and polar organic solvents.

8.2.2 Form

Refer to the FDA package insert.

8.2.3 Storage and Stability

Refer to the FDA package insert.

8.2.4 Handling

Qualified personnel, familiar with procedures that minimize undue exposure to themselves and the environment, should undertake the preparation, handling, and safe disposal of the chemotherapeutic agent in a self-contained and protective environment.

8.2.5 Availability

Gemcitabine is a commercially available agent. The cost of gemcitabine will be billed to participant insurance.

8.2.6 Administration

Refer to **Section 5**.

8.2.7 Ordering

Gemcitabine is a commercially available agent that will be ordered by site pharmacy personnel per local standards of practice.

8.2.8 Accountability

Gemcitabine accountability will be handled in accordance with local institutional standards of

practice.

8.2.9 Destruction and Return

Expired or unused supplies of gemcitabine should be destroyed according to institutional policies.

9. BIOMARKER, CORRELATIVE, AND SPECIAL STUDIES

9.1 Tumor Biopsy Collection

Core needle, incisional, or excisional biopsies may be obtained for analysis. Biopsy procedures will be performed following standard institutional procedures. The tissue collection goal is 3 – 6 core biopsy samples collected using an 18 - 20 gauge needle. Less than the goal amount of tissue is acceptable and should be based upon the clinical judgment of the treating investigator and the clinician performing the procedure. In the event tissue is insufficient for analysis it will not be considered a protocol violation.

- A mandatory pretreatment fresh tumor tissue biopsy will be obtained from all participants prior to starting gemcitabine/Azenosertib
    - If tissue is inadequate or unavailable for research testing, archival tissue will be requested for subjects who consent to the optional use of their samples.
- A mandatory on-treatment biopsy will occur on cycle 1, day 9, 10, or 11. If the biopsy cannot be obtained during the first cycle,it will be obtained on cycle 2, day 9, 10, or 11.
- An additional optional biopsy at the time of acquired resistance will be offered to all participants who experienced clinical benefit on trial as judged by the treating investigator.
    - The time of resistance biopsy should be obtained prior to the initiation of another anti-cancer therapy when possible. In the event this is not feasible, the time of resistance biopsy may be obtained up to 30 days after the date that disease progression was determined. The date of progression may be considered either the date the radiological scan was performed, or the date the treating investigator judged the participant to have progressed.

Please refer to the protocol laboratory manual for specific handling and shipping instructions.

9.2 Germline Blood Sample Collection

For performance of WES, a single germline blood sample will be collected from all participants. The blood sample will be collected during any study visit (preferably during screening or cycle 1).

A total of 10 mL of blood will be collected for germline testing.

Please refer to the protocol laboratory manual for specific collection and handling instructions.

9.3 Collection of cfDNA and PBMCs

Serial blood sampling will be performed for cfDNA and PBMC collection on all patients.

Please refer to the protocol laboratory manual for specific collection and handling instructions.

10. STUDY CALENDAR

Screening evaluations are to be conducted within 14 days prior to the start of protocol therapy, with the exception of obtaining the informed consent, pre-treatment biopsy, and imaging, which may be obtained up to 30 days prior to the start of protocol therapy. Assessments must be performed prior to administration of the study agent.

	Screening	Cycle 1 Day 1[a]	Cycle 1 Day 8[b]	Cycle 1 Day 9	Cycle 1 Day 10	Cycle 1 Day 11	Cycle 1 Day 15	Cycles 2+ Day 1[c]	Cycles 2+ Day 8[b]	Cycle 2 Day 9	Cycle 2 Day 15	Off Treatment[d]	Every 2 Months Following Treatment Discontinuation	EDC Timepoints[¥]
Informed consent	X													N/A
Demographics	X													Screening
Medical history	X													Screening
Physical exam[q]	X	X	X				X	X			X	X		N/A
Vital signs[e, q]	X	X	X				X	X	X		X	X		N/A
Height	X													N/A
Weight	X	X	X				X	X	X		X	X		N/A
ECOG performance status	X	X					X	X			X	X		Screening, Day 1 of every cycle, Off Treatment
CBC w/diff, plts	X	X	X				X	X	X		X	X		N/A
Serum chemistry[f]	X	X	X				X	X	X		X	X		N/A
Tumor markers[r]		X						X				X		
Urine or Serum β-HCG[g]	X	X						X						N/A
ECG[h]	X	X						X						N/A
Adverse event evaluation[q]		X----------										X		All visits
Radiological evaluation[i]	X	Radiological measurements should be performed every 8 weeks (+/- 7 day scheduling window)										X		Screening, Every 8 weeks, Off Treatment
Tumor Biopsy[j]	X			X[j]	X[j]	X[j]				X[j]		X		Screening, On-Treatment, Off Treatment

	Screening	Cycle 1 Day 1[a]	Cycle 1 Day 8[b]	Cycle 1 Day 9	Cycle 1 Day 10	Cycle 1 Day 11	Cycle 1 Day 15	Cycles 2+ Day 1[c]	Cycles 2+ Day 8[b]	Cycle 2 Day 9	Cycle 2 Day 15	Off Treatment[d]	Every 2 Months Following Treatment Discontinuation	EDC Timepoints[¥]
cfDNA[k]	X	X	X					X				X		Screening, **Days 1 and 8 of cycle 1.** Day 1 of all cycles, Off Treatment
PBMC[k]	X	X						X[k]				X		Screening, Day 1 of cycle 1, **Day 1 of even cycles after cycle 2,** Off Treatment
Germline Blood Sample[l]		X												
Azenosertib		As described in **Section 5.3**												
Gemcitabine		X	X					X	X					Day 1, day 8 of every cycle
Viral Hepatitis Monitoring[n]	X													
Telephone or Care Provider Contact[m]						X[o]							X	Every 2 months following treatment discontinuation
Archival Tissue[p]	X													

	Screening	Cycle 1 Day 1[a]	Cycle 1 Day 8[b]	Cycle 1 Day 9	Cycle 1 Day 10	Cycle 1 Day 11	Cycle 1 Day 15	Cycles 2+ Day 1[c]	Cycles 2+ Day 8[b]	Cycle 2 Day 9	Cycle 2 Day 15	Off Treatment[d]	Every 2 Months Following Treatment Discontinuation	EDC Timepoints[¥]

¥: Note: column relevant only for CRF builders.

a. Please refer to **Section 5.2.1**.
b. A -1/+3 day scheduling window is allowable to account for holidays, adverse weather, vacations, or any other scheduling issues. When the dosing window is used to move the date of gemcitabine, dosing of Azenosertib is also adjusted so that Azenosertib is always administered on the five days following each gemcitabine infusion.
c. A ± 7 day scheduling window is allowable to account for holidays, adverse weather, vacations, or any other scheduling issues.
d. Off treatment evaluation up to 30 days after end of treatment. Note: for IND trials, follow up visits or other contact are required in order to identify SAEs during the 30 days following the end of study treatment.
e. Heart rate, respiratory rate, temperature, blood pressure, and oxygen saturation; to be performed prior to study agent dosing on applicable visit days.
f. Albumin, alkaline phosphatase, total bilirubin, bicarbonate, BUN, calcium, chloride, creatinine, glucose, potassium, total protein, SGOT [AST], SGPT [ALT], sodium, creatinine. Labs should be reviewed and approved by the Investigator or qualified designee prior to Azenosertib dosing at each visit in which labs are collected. Once approved, a member of the study team should contact the subject to confirm Azenosertib should be taken.
g. Serum or urine test to be collected within 7 days of the first dose of study agent, at Day 1 of each cycle, and when indicated by symptomology. Serum or urine pregnancy test only required for women of child-bearing potential. Childbearing potential defined per **Section 3.1.10.**
h. Single ECG to be collected during screening and day 1 of each cycle for QTc monitoring.
i. Radiologic imaging of any disease-involved site**.** Confirmatory scans must also be obtained not less than 4 weeks following initial documentation of an objective response (PR or CR).
j. A tumor biopsy is mandatory at baseline for all participants, and mandatory on either Cycle 1 Day 9-11 (preferred) **OR** Cycle 2 Day 9-11 if the biopsy could not be obtained on Cycle 1 Day 9-11. An additional optional biopsy at the time of acquired resistance will be offered to all participants who experienced clinical benefit on study as judged by the treating investigator. Tumor biopsies to be collected as per **Section 9.1**.
k. Blood samples to be collected as per **Section 9.3**. Refer to the laboratory manual for specific collection and handling instructions.
l. A **single** germline blood sample to be collected from all participants preferably during screening or cycle 1 (however may be collected at any study visit). See **Section 9.2**. Refer to the laboratory manual for specific collection and handling instructions.
m. Following treatment discontinuation, participants will continue to be followed for survival status and disease response (as applicable if study treatment was discontinued prior to progression) until death. No imaging or study visits will be mandated as part of this follow up, but disease response will be captured by review of any available imaging, medical records, and/or healthcare provider exams performed as part of standard of care. Survival status may be verified via medical record review, contact with care providers, or telephone contact with the patient. A ±14 day window is permitted.
n. Viral hepatitis monitoring is only required in patients who have baseline viral hepatitis. Viral hepatitis monitoring is performed by checking the viral load. Viral hepatitis monitoring should be performed, when appropriate (see above), every 2 cycles starting in cycle 2.
o. Study team phone call to determine if dose hold is needed. Emphasize activity and exercise to prevent fatigue.
p. Optional archival tissue collection. Refer to the laboratory manual for specific collection and handling instructions.

	Screening	Cycle 1 Day 1[a]	Cycle 1 Day 8[b]	Cycle 1 Day 9	Cycle 1 Day 10	Cycle 1 Day 11	Cycle 1 Day 15	Cycles 2+ Day 1[c]	Cycles 2+ Day 8[b]	Cycle 2 Day 9	Cycle 2 Day 15	Off Treatment[d]	Every 2 Months Following Treatment Discontinuation	EDC Timepoints[¥]
				q. All participants should be monitored carefully for any signs of infection (including, but not limited to, temperature ≥99.5°F/37.5°C, unexplained tachycardia, increased sweating, chills, cough, urinary symptoms suggestive of a urinary tract infection, diarrhea, or unusual abdominal pain) even in the absence of neutropenia and to perform an appropriate fever work up (including but not limited to CBC with differential, complete metabolic panel, urinalysis and culture, and other tests such as chest x-ray per symptomatology). In the event an infection is suspected, treatment with empiric antibiotics should be administered while waiting for culture results to be returned. r. CA19-9 and CEA blood tests drawn on day 1 of each cycle and end of treatment.											

11. MEASUREMENT OF EFFECT

11.1 Antitumor Effect – Solid Tumors

For the purposes of this study, participants should be re-evaluated for response every 8 weeks (± 7 day scheduling window). In addition to a baseline scan, confirmatory scans should also be obtained not less than 4 weeks following initial documentation of objective response.

Patients who discontinue protocol treatment prior to disease progression will continue to undergo restaging scans as clinically indicated until the documentation of disease progression.

Response and progression will be evaluated in this study using the new international criteria proposed by the Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1).[38] Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

11.1.1 Definitions

Evaluable for Target Disease response. Only those participants 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 target disease response. These participants will have their response classified according to the definitions stated below. (Note: Participants who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

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

11.1.2 Disease Parameters

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

Note: Tumor lesions that are situated in a previously irradiated area will not be considered measurable unless there has been demonstrated progression in the lesion.

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

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

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

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

11.1.3 Methods for Evaluation of Disease

All measurements should be taken and recorded in metric notation using a ruler, calipers, or a digital measurement tool. 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 in 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 thickness is 5mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size of 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.

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

(a) Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.

(b) No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.

(c) FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be

prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

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

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.

MIBG (meta-iodobenzylguanidine). The following is recommended, to assure high quality images are obtained.

Patient preparation: Iodides, usually SSKI (saturated solution of potassium iodide), are administered to reduce thyroidal accumulation of free radioiodine, preferably beginning the day prior to injection and continuing for 3 additional days (4 days total). For infants and children, one drop t.i.d. is sufficient, for adolescents 2 drops t.i.d., and for adults 3 drops t.i.d. Participants and/or parents are asked about exposure to potential interfering agents. If none is noted, an indwelling intravenous line is established. The dose of MIBG is administered by slow intravenous injection over 90 seconds.

Images from the head to the distal lower extremities should be obtained.

I-123MIBG scintigraphy is performed to obtain both planar and tomographic images.

Planar: Anterior and posterior views from the top of the head to the proximal lower extremities are obtained for 10 minutes at 24 hours and occasionally at 48 hours following injection of 10 mCi/1.7 square meters of body surface area (~150 μCi/kg, maximum 10 mCi). Anterior views of the distal lower extremities are adequate. A large field of view dual head gamma camera with low energy collimators is preferred.

SPECT: Most participants receiving I-123 MIBG also undergo SPECT at 24 hours, using a single or multi-headed camera with a low energy collimator. The camera is rotated through 360 degrees, 120 projections at 25 seconds per stop. Data are reconstructed using filtered back projections with a Butterworth filter and a cut off frequency of 0.2-0.5. SPECT/CT may be performed at institutions with this capacity.

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 data 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 from 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 participant 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.

11.1.3.1 Evaluation of Target Lesions

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

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

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

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

11.1.3.2 Evaluation of Non-Target Lesions

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

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

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

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

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

11.1.3.3 Evaluation of New Lesions

The finding of a new lesion should be unequivocal (i.e. not due to difference in scanning technique, imaging modality, or findings thought to represent something other than tumor (for example, some 'new' bone lesions may be simply healing or flare of pre-existing lesions). However, a lesion identified on a follow-up scan in an anatomical location that was not scanned at baseline is considered new and will indicate PD. If a new lesion is equivocal (because of small size etc.), follow-up evaluation will clarify if it truly represents new disease and if PD is confirmed, progression should be declared using the date of the initial scan on which the lesion was discovered.

11.1.3.4 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.

Table 14: For Participants 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: Participants 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.

Table 15: For Participants with Non-Measurable Disease (i.e., Non-Target Disease)		
Non-Target Lesions	**New Lesions**	**Overall Response**
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

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

11.1.4 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, or death due to any cause. Participants without events reported are censored at the last disease evaluation).

Duration of overall complete response: 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, or death due to any cause. Participants without events reported are censored at the last disease evaluation.

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.

11.1.5 Survival Outcomes

Overall Survival: Overall Survival (OS) is defined as the time from randomization (or registration) to death due to any cause, or censored at date last known alive.

Progression-Free Survival: Progression-Free Survival (PFS) is defined as the time from randomization (or registration) to the earlier of progression or death due to any cause. Participants alive without disease progression are censored at date of last disease evaluation.

Time to Progression: Time to Progression (TTP) is defined as the time from randomization (or registration) to progression, or censored at date of last disease evaluation for those without progression reported.

11.1.6 Response Review

Confirmation of scan results will be done centrally using the Tumor Imaging Metrics Core (TIMC) at DF/HCC, however treatment decisions may be made based on local scan interpretation.

12. DATA REPORTING / REGULATORY REQUIREMENTS

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

12.1 Data Reporting

12.1.1 Method

The Office of Data Quality (ODQ) will collect, manage, and perform quality checks on the data for this study.

12.1.2 Responsibility for Data Submission

Investigative sites within DF/HCC or DF/PCC are responsible for submitting data and/or data forms to the Office of Data Quality (ODQ) in accordance with DF/HCC policies.

12.2 Data Safety Monitoring

The DF/HCC Data and Safety Monitoring Committee (DSMC) will review and monitor toxicity and accrual data from this study. The committee is composed of medical oncologists, research nurses, pharmacists and biostatisticians with direct experience in cancer clinical research. Information that raises any questions about participant safety will be addressed with the sponsor-investigator and study team.

The DSMC will review each protocol up to four times a year with the frequency determined by the outcome of previous reviews. Information to be provided to the committee may include: up-to-date participant accrual; current dose level information; DLT information; all grade 2 or higher unexpected adverse events that have been reported; summary of all deaths occurring within 30 days of intervention for Phase I or II protocols; for gene therapy protocols, summary of all deaths while being treated and during active follow-up; any response information; audit results, and a summary provided by the study team. Other information (e.g. scans, laboratory values) will be provided upon request.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/Endpoints

This is a proof of concept phase 2 clinical trial with safety lead-in that is testing the Azenosertib WEE1 inhibitor combined with gemcitabine in platinum-resistant advanced pancreatic cancer.

13.1.1 Safety Lead-In

A minimum of 6 patients and up to 12 patients will be enrolled in a safety lead-in cohort to evaluate the safety of the combination of Azenosertib and gemcitabine. Gemcitabine will be held at a fixed dose of 800 mg/m^2 on days 1 and 8 while the starting dose of Azenosertib will be 150 mg once daily (dose level 1 [DL1]) on days 1-5, days 8-12, and days 15-19. Following a 3+3 algorithm, an initial 3 patients will be enrolled at DL1. If 0 or 1 of 3 patients experience a DLT, an additional 3 patients will be enrolled to DL1. DL1 will be declared the confirmed RP2D (RP2D$_c$) if at most 1 of 6 patients treated at DL1 experience a DLT. Alternatively, if there are 2 or more DLTs observed at DL1, 3 patients will be enrolled at DL -1 (Gemcitabine will be given at 600 mg/m^2 on days 1 and 8 of a 21-day cycle) and the 3+3 rules repeated for safety evaluation. The RP2D$_c$ will be the highest DL at which at most 1 patient of 6 experiences a DLT. If DL -1 is considered too toxic, the combination will be considered unsafe for further development at the explored dose levels.

Participants enrolled to the safety run-in portion of the trial will be required to have received at least 75 percent of their assigned doses of Azenosertib during the first cycle to be considered evaluable for DLT purposes. Participants will be replaced if they do not meet these parameters during cycle 1 for reasons other than toxicity (e.g. withdrawal of consent for participation, or rapid disease progression and subsequent removal from the trial).

13.1.2 Phase 2

Once the $RP2D_c$ has been determined, we will investigate the efficacy of therapy and will include patients treated with the $RP2D_c$ in the phase 2 analysis. We will use a Simon two-stage minimax design to evaluate efficacy where the null hypothesis is that the true 6-month PFS is ≤ 24% (unacceptable historical control data) and the alternative hypothesis is that the true 6-month PFS is ≥ 44%. Participants who are alive without disease progression and remain on trial therapy for at least 6 months from registration will be considered to have achieved PFS ≥ 6 months. Participants who have disease progression, are deceased, or discontinue trial therapy within 6 months from registration will be considered to have PFS < 6 months. In stage 1, 23 patients will be enrolled; all patients who were treated in the safety lead-in will be included in stage 1. If 6-month PFS rate is ≤ 21.7% (≤ 5/23 patients with at least 6-month PFS), enrollment will end, and the combination will be declared futile. If 6 or more patients have ≥ 6-month PFS, then an additional 11 patients will be enrolled for a total sample size of 34. The combination will be considered promising if 6-month PFS rate is ≥13/34 patients. This design has a 5% chance of incorrectly declaring promise (one-sided α=0.05), and an 80% chance of correctly declaring promise (power = 0.8). The probability of early termination under the null hypothesis is 0.51.

13.2 Interim analyses

13.2.1 Futility stopping

There will be an interim futility analysis after 23 patients are enrolled as required by the Simon two-stage design.

13.2.2 Safety monitoring

Bayesian toxicity monitoring will be used to perform continuous safety monitoring. Specifically, patients will be monitored for incidence of serious adverse events (SAEs) as described in **Section 7.3**. We assume the true SAE rate follows a beta (0.5, 0.5) prior distribution and, conditional on the true SAE rate, the number of patients who experience an SAE follows a binomial distribution. If the posterior Prob (SAE rate > 33%) > 50%, trial enrollment will be paused. (Note: Bayesian toxicity monitoring parameters were selected to calibrate with the 3+3 design in safety lead-in.) If the trial is paused, the safety review committee will review the totality of the safety data and determine whether to continue patient enrollment at the current dose levels, introduce intermediate or lower dose levels for evaluation, or close the trial to accrual and declare the combination too toxic. This monitoring rule has a 6.1% and 99.5% probability of pausing if the true DLT-E rate is 10% or 50%, respectively. The table below specifies safety monitoring thresholds.

Table 16: Bayesian toxicity monitoring boundaries		
No. of patients	**No. of DLT-Es to pause study**	**Observed DLT-E rate**
7-9	≥3	≥0.33
10-12	≥4	≥0.33
13-15	≥5	≥0.33
16-18	≥6	≥0.33
19-21	≥7	≥0.33
22-24	≥8	≥0.33
25-27	≥9	≥0.33
28-30	≥10	≥0.33
31-33	≥11	≥0.33
34 – cohort fully accrued		

13.3 Final analyses

For the primary endpoint, the 6-month PFS rate will be calculated as a binary outcome. Participants who are alive without disease progression and remain on trial therapy for at least 6 months from registration will be considered to have achieved PFS ≥ 6 months. Participants who have disease progression, are deceased, or discontinue trial therapy within 6 months from registration will be considered to have PFS < 6 months. Secondary objectives include the determination of the objective response rate (ORR), duration of response (DoR), disease control rate (DCR), median PFS and OS. PFS is defined as the time from registration to documented disease progression or death from any cause, whichever occurs first. Subjects who have not experienced an event of interest by the time of analysis will be censored at the date of the last disease assessment without progression. OS is defined as the time from registration to death from any cause, and subjects who are thought to be alive at the time of final analysis will be censored at the last date of contact. Tumor reassessment with baseline CT scan and restaging scans will occur every 8-10 weeks using RECIST v1.1.

The safety of the gemcitabine/Azenosertib combination will be followed and toxicities will be reported using CTCAE version 5.0.

Time-to-event distributions will be estimated using the Kaplan-Meier method. Comparisons of subgroups will be made using the log rank test and Cox modeling. Response and toxicity rates will be compared between subgroups using Fisher's exact tests; multivariable logistic regression modeling will be used to adjust for the effect of any covariates that are associated with these categorical outcomes.

13.4 Sample Size, Accrual Rate and Study Duration

The planned sample size is a maximum of 34 subjects. The planned accrual rate across DF/HCC 2 patients per month, for a total accrual duration of approximately 16 months. For determination of the primary endpoint, the last participant accrued will need to be observed for at least 6 months following the initiation of study treatment, for an estimated duration to primary completion of 24 months. Participants will be observed until death for survival status and disease

response (as applicable).

Accrual Targets								
Ethnic Category	**Sex/Gender**							
	Females			**Males**			**Total**	
Hispanic or Latino	2		+	2		=	4	
Not Hispanic or Latino	15		+	15		=	30	
Ethnic Category: Total of all subjects	17 (A1)		+	17	(B1)	=	34	(C1)
Racial Category								
American Indian or Alaskan Native	0		+	0		=	0	
Asian	2		+	2		=	4	
Black or African American	2		+	2		=	4	
Native Hawaiian or other Pacific Islander	0		+	0		=	0	
White	13		+	13		=	26	
Racial Category: Total of all subjects	17	(A2)	+	17	(B2)	=	34	(C2)
	(A1 = A2)			(B1 = B2)			(C1 = C2)	

13.2.1 Planned Efforts to Reach Accrual Targets

The study will reflect the patient population at the enrolling sites.

13.2.2 Efforts to Support Retention and Resource Needs of Participants

The therapy schedule has flexible time windows.

13.2.3 Tracking decline of consent and participant withdrawal

Patient withdrawal will be tracked in the database to monitor the diversity of sample size and accrual rate.

13.5 Analysis of Exploratory Correlative Objectives

We will fully characterize the DNA repair status of the pre-treatment and on-treatment biopsies at both the genomic and functional levels. WES and RNAseq will be performed, with computational analyses focusing on alterations in DNA repair pathway genes and expression levels of these genes.

The genomic and functional characterization of biopsies and cfDNA will be correlated with clinical outcome (i.e. complete or partial response, stable disease or progressive disease) defined by RECIST 1.1 including DDR gene and P53 mutations. We will use logistic regression to investigate associations with the binary outcome of 6-month PFS rate and Cox proportional hazards regression for the time to event analyses of PFS, with adjustment for relevant covariates

including age, gender, race/ethnicity, ECOG performance status, and metastatic sites.

13.5.1 Proof of Mechanism testing

Having paired pre-treatment and on-treatment biopsies will allow us to test our hypothesis that the gemcitabine/Azenosertib combination increases replicative stress as proof-of-mechanism. To evaluate this hypothesis, we will examine gene expression by RNA-seq as well as levels of replication stress, ATR activity and DNA damage biomarkers by IHC, including pKAP1, pRPA32, Cyclin E, MYC, pATR, pCHK1 and γ-H2AX. Additionally, DNA fiber assays will be used to assess if gemcitabine/Azenosertib combination treatment converts a pancreatic cancer with stable replication forks to one with unstable replication forks.

13.5.2 Resistance testing

Biopsies at the time of tumor resistance will be descriptive in nature, noting secondary genomic alterations or gene expression changes compared to pre-treatment samples. Because SLFN11 deficiency has been correlated with gemcitabine resistance, we will use IHC assays to evaluate SLFN11 protein levels. Using biopsies from our gemcitabine/Azenosertib treated patients, we will assess if SLFN11 is predictive of sensitivity to this combination in pancreatic cancer patients.

13.5.3 Tumor microenvironment

Given the potential role of WEE1 inhibition and immune modulation of the tumor immune microenvironment, effects on the TiME will be assessed using a combination of flow cytometry, multiplexed immunofluorescence, and spatial transcriptomics to discover novel mechanisms of effectiveness and resistance.

13.5.4 Cell free DNA (cfDNA)

cfDNA will be used to evaluate whether there are genomic alterations that may be associated with improved clinical outcome with gemcitabine/Azenosertib and to assess for mechanisms of resistance.

13.5.5 Organoid culture

Biopsies will also be used for generation of organoid cultures. Organoid cultures will be used for therapeutic drug testing with the gemcitabine/Azenosertib combination, as well as other DNA repair inhibitors, assayed by Cell Titer Glo to correlate with clinical responses.

13.5.6 Peripheral blood mononuclear cells (PBMCs)

Given the potential role of WEE1 inhibition in immune modulation, effects on the circulating peripheral blood mononuclear cells will be banked for future analyses to discover novel mechanisms of effectiveness and resistance.

13.6 Reporting and Exclusions

13.6.1 Evaluation of Toxicity

All participants will be evaluable for toxicity from the time of their first dose of study drug.

13.6.2 Evaluation of the Primary Efficacy Endpoint

All eligible patients included in the study who receive study medication will be assessed for 6-month PFS, even if there are major protocol therapy deviations.

14. PUBLICATION PLAN

The results should be made public within 24 months of reaching the end of the study. The end of the study is the time point at which the last data items are to be reported, or after the outcome data are sufficiently mature for analysis, as defined in the section on Sample Size, Accrual Rate and Study Duration. If a report is planned to be published in a peer-reviewed journal, then that initial release may be an abstract that meets the requirements of the International Committee of Medical Journal Editors. A full report of the outcomes should be made public no later than three (3) years after the end of the study.

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APPENDIX A PERFORMANCE STATUS CRITERIA

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

APPENDIX B CLASSIFICATION OF SUBSTRATES, INHIBITORS, AND INDUCERS OF CYTOCHROME P450 ENZYMES AND TRANSPORTERS

The classification included in this section is based on the Food and Drug Administration (FDA) Guidance for Industry, Clinical Drug Interaction Studies — Cytochrome P450 Enzyme- and Transporter-Mediated Drug Interactions (CDER 2020).

Azenosertib's actions as a substrate, inhibitor, and inducer include:

- Azenosertib is a substrate for CYP3A4 and P-gp. Therefore, strong and moderate CYP3A4 inhibitors and inducers as well as P-gp inhibitors are prohibited.
- Azenosertib is a potent inhibitor of MATE1 and MATE2K, so subjects who are taking substrates of MATE1 and MATE2K should be carefully monitored for AEs that may be related to drug-drug interactions.
- Azenosertib is not a potent inhibitor of other transporters and CYPs. It is however recommended to closely monitor subjects who are taking substrates of CYP3A4, P-gp and BCRP.
- Azenosertib is an inducer of CYP2B6, so subjects who are taking CYP2B6 substrates will also need to be monitored closely.

Examples of CYP3A inhibitors include:

Cytochrome P450 (CYP) Enzymes	**Strong Inhibitors**[a] **≥5-fold-increase in AUC or >80% decrease in CL**	**Moderate inhibitors**[b] **≥2 but <5-fold-increase in AUC or 50-80% decrease in CL**
CYP3A	Boceprevir, clarithromycin, cobicistat, conivaptan, danoprevir and ritonavir, diltiazem, elvitegravir and ritonavir, grapefruit juice[c], indinavir and ritonavir, idelalisib, itraconazole, ketoconazole, lopinavir and ritonavir, nefazodone, nelfinavir, paritaprevir and ritonavir and (ombitasvir and/or dasabuvir), posaconazole, ritonavir, saquinavir and ritonavir, telaprevir, tipranavir and ritonavir, troleandomycin, voriconazole	Cimetidine, ciprofloxacin, clotrimazole, crizotinib, cyclosporine, dronedarone, erythromycin, fluconazole, fluvoxamine, imatinib, tofisopam, verapamil

[a] A strong inhibitor for a specific CYP is defined as an inhibitor that increases the area under the concentration-time curve (AUC) of a substrate for that CYP by equal to or more than 5-fold.

[b] A moderate inhibitor for a specific CYP is defined as an inhibitor that increases the AUC of a sensitive substrate for that CYP by less than 5-fold but equal to or more than 2-fold.

[c] The effect of grapefruit juice varies widely among brands and is concentration-, dose-, and preparation-dependent. Studies have shown that it can be classified as a "strong CYP3A inhibitor" when a certain preparation was used

(e.g., high dose, double strength) or as a "moderate CYP3A inhibitor" when another preparation was used (e.g., low dose, single strength).

Examples of CYP3A inducers include:

Cytochrome P450 (CYP) Enzymes	**Strong Inducers[a] ≥80% decrease in AUC**	**Moderate Inducers[b] 50-80% decrease in AUC**
CYP3A	carbamazepine, enzalutamide, mitotane, phenytoin, rifampin, St. John's wort[c]	bosentan, efavirenz, etravirine, modafinil

[a] A strong inducer is a drug that decreases the AUC of sensitive index substrates of a given metabolic pathway by ≥80%.
[b] A moderate inducer is a drug that decreases the AUC of sensitive index substrates of a given metabolic pathway by ≥50% to <80%.
[c] The effect of St. John's wort varies widely and is preparation-dependent.

Examples of P-gp inhibitors include:

Transporters	**Gene**	**Inhibitor**
P-gp[a]	ABCB1	amiodarone, carvedilol, clarithromycin, dronedarone, itraconazole, lapatinib, lopinavir and ritonavir, propafenone, quinidine, ranolazine, ritonavir, saquinavir and ritonavir, telaprevir, tipranavir and ritonavir, verapamil

[a] P-gp: (1) AUC fold-increase of digoxin ≥2 with co-administration and (2) in vitro inhibitor.

Examples of CYP3A substrates include:

Cytochrome P450 (CYP) Enzymes	**Sensitive Substrates[a]**	**Moderate Sensitive Substrates[b]**
CYP3A	alfentanil, avanafil, budesonide, buspirone, conivaptan, darifenacin, darunavir, dasatinib, dronedarone, ebastine, eletriptan, eplerenone, everolimus, felodipine, ibrutinib, indinavir, lomitapide, lovastatin, lurasidone, maraviroc, midazolam, naloxegol, nisoldipine, quetiapine, saquinavir, sildenafil, simvastatin, sirolimus, tacrolimus, ticagrelor, tipranavir, tolvaptan, triazolam, vardenafil	alprazolam, atorvastatin, colchicine, eliglustat, pimozide, rilpivirine, rivaroxaban, tadalafil

[a] Sensitive substrates are drugs that demonstrate an increase in area under the concentration-time curve (AUC) of ≥5-fold with strong index inhibitors of a given metabolic pathway in clinical drug-drug interaction studies.

[b] Moderate sensitive substrates are drugs that demonstrate an increase in AUC of ≥2 to <5-fold with strong index inhibitors of a given metabolic pathway in clinical drug-drug interaction studies.

Substrates for CYP2B6 are not listed because per the FDA guidelines, they currently do not have index substrates for this enzyme.

Example of substrates for transporters include:

Transporters	Gene	Substrate
P-gp[a]	ABCB1	Dabigatran, digoxin, fexofenadine
BCRP[b]	ABCG2	Rosuvastatin, sulfasalazine
MATE1/MATE2K	SLC47A1, SLC47A2	Metformin

[a] P-gp: (1) AUC fold-increase ≥2 with verapamil or quinidine co-administration and (2) in vitro transport by P-gp expression systems, but not extensively metabolized.

[b] BCRP: (1) AUC fold-increase ≥2 with pharmacogenetic alteration of ABCG2 (421C>A) and (2) in vitro transport by BCRP expression systems.