

A DOSE ESCALATION TRIAL OF THE Wee1 INHIBITOR AZD-1775, IN COMBINATION WITH GEMCITABINE (+RADIATION) FOR PATIENTS WITH UNRESECTABLE ADENOCARCINOMA OF THE PANCREAS

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**SYNOPSIS****TITLE**

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A Dose Escalation Trial of AZD-1775 (a Wee1 inhibitor) and Gemcitabine-Radiation in Locally Advanced Pancreatic Cancer

**PROTOCOL NUMBER**

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UMCC 2013.094

**CLINICAL PHASE**

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Phase I/II

**RATIONALE**

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We have developed the use of the combination of gemcitabine and radiation in the treatment of locally advanced pancreatic cancer. These agents cause DNA damage to cancer cells leading to tumor response. However, the disease often progresses after treatment. Our preclinical studies at the University of Michigan have shown that normal cells have both a functional G1 and G2 checkpoint that permits them to repair DNA damage before undergoing DNA synthesis and mitosis, respectively, thereby avoiding cells death. In contrast, pancreatic cancer cells lack a G1 checkpoint after DNA damage due either to mutations in p53 or in the p53 pathway. Our preclinical work has demonstrated that inhibition of Wee1 kinase by AZD-1775 abrogates the G2 checkpoint, the only functional checkpoint in pancreatic cancer cells, which causes the pancreatic cancer cells, but not normal cells, to progress into mitosis before repairing the DNA damage, leading to pancreatic cancer cell death. Pancreatic cancer cells are selectively killed compared to normal cells, which retain a functional G1 checkpoint. AZD-1775 causes a selective increase in pancreatic cancer cell death both from radiation and from gemcitabine, suggesting that this strategy will improve the outcome of treatment of both local and (occult) systemic disease.

**OBJECTIVES**

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The objectives of this study are:

*Primary*

Determine the maximum tolerated dose of AZD-1775 in combination with gemcitabine and radiation in patients with locally advanced pancreatic cancer.

*Secondary*

1. To estimate the efficacy of this regimen (combined with standard systemic therapy) at the target dose, as determined by progression-free survival and overall survival at one year.
2. To determine if Wee1 is inhibited by AZD-1775 at or below its target dose in surrogate tissues.

## **STUDY DESIGN**

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Protocol therapy will consist of administration of both AZD-1775 and gemcitabine (with and without radiation) at assigned dose levels in accordance with a Time-to-Event Continual Reassessment Method (TITE-CRM).

1. Cycle 1: AZD-1775 will be given orally (day 1,2 and 8,9 of a 21-day cycle) with gemcitabine given intravenously over 30 minutes on day 1 and day 8.
2. Cycles 2 and 3: The same 21-day cycle of AZD-1775 and gemcitabine will be administered as in Cycle 1 but with concurrent radiation therapy. Two cycles will be given with 25 radiation treatments.
3. Cycle 3 is followed by a 3 week break in treatment.
4. Cycle 4: AZD-1775-gemcitabine as described in cycle 1.

The maximum tolerated dose (MTD) will be determined based on the development of dose-limiting toxicities within the first 105 days of therapy.

## **NUMBER OF PATIENTS**

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Approximately 36 patients will be accrued to this study.

## **TARGET POPULATION**

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Patients must have pathologically confirmed locally advanced pancreatic carcinoma.

## **ESTIMATED LENGTH OF PATIENT PARTICIPATION**

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Patients may continue to receive treatment until the development of unacceptable toxicities or progressive disease. It is estimated that patients who discontinue treatment because of toxicity or progressive disease will participate an average of 3 months with one additional month of follow-up. Patients who experience a response or stable disease are expected to participate an average of 6 months plus an additional 6-12 months for follow-up.

## **ESTIMATED STUDY DATES**

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December 2013 (first study screening visit) to December 2018 (last study follow-up visit)

## **INVESTIGATIONAL REGIMEN DOSE/ ROUTE/ DURATION**

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AZD-1775 will be administered at assigned dose and taken orally on days 1 and 2 and on days 8 and 9 until the development of unacceptable toxicity, progression of disease, or patient decision to discontinue therapy.

## **COMBINATION TREATMENT(S)**

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Gemcitabine and radiation therapy

## **SAFETY ASSESSMENTS**

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Safety assessments will be conducted every weekly on the day of chemotherapy administration and weekly during the course of radiation therapy and include clinical evaluation, assessment of adverse events and laboratory evaluation.

Adverse event seriousness, severity grade, and relationship to study treatment will be assessed by the investigator. Severity grade will be defined by the National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 (to permit comparison to prior studies of AZD-1775).

## **TUMOR ASSESSMENTS**

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Tumor evaluation using CT or MRI will be performed within 4 weeks of registration and prior to beginning cycle 4 of treatment (1 month after chemoradiation) and approximately every 3 months afterwards. Response will be evaluated using Response Evaluation Criteria in Solid Tumors (RECIST) v.1.1.

## **OTHER ASSESSMENTS**

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During the first or second cycle of treatment, patients will be asked to undergo 2 punch biopsies of the skin: one will occur approximately 3 h after treatment with gemcitabine (but before AZD-1775) on day 1 of a cycle and the second will occur on day 2 of a cycle approximately 3-5 hours after AZD-1775. Utilizing immunohistochemistry, we will assess Wee1 signaling in response to AZD-1775 in combination with gemcitabine. Using a punch biopsy of the skin to evaluate replicating cells in hair follicles, we anticipate that AZD-1775 plus gemcitabine will reduce Cdk1 (Y15) phosphorylation relative to gemcitabine alone, confirming Wee1 inhibition. To confirm Wee1 pathway inhibition, we will assess the mitotic marker pHistoneH3, and anticipate that it will be increased in response to AZD-1775 plus gemcitabine relative to gemcitabine alone. Other potential biomarkers of Wee1 inhibition will also be assessed.

## **STATISTICAL METHODS**

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The trial will be monitored using a modification of the Continual Reassessment Method, called TITE-CRM. The TITE-CRM method assumes a model for the time to occurrence of toxic response as a function of dose, and allows information from all patients enrolled in the trial to be employed when allocating a new patient to a dose level. Because this method is very flexible in terms of the number of patients treated at each dose, patients may be continuously recruited throughout the trial, without recruitment pauses, as long as patients are treated at a dose consistent with the current safety profile.

The acute observation period for toxicity is defined as the first 4 cycles of treatment (with a 3 week break between cycle 3 and cycle 4), for a total of 105 days in length.

The target rate DLT is 30%. The target rate will define the MTD of AZD-1775) given concurrently with gemcitabine (and radiation therapy for 2 cycles) for locally advanced pancreatic cancer.

Expected rates of acute toxicity have been estimated based upon our previous treatment experience with gemcitabine-radiation plus cisplatin or oxaliplatin and from gemcitabine-AZD-1775 in advanced pancreatic cancer patients and clinically acceptable maximal

levels of toxicity. These rates will be re-estimated throughout the conduct of this trial as treatment experience is accrued.

Sample Size

This study will enroll 36 patients for the estimation of the dose-toxicity function. We expect to accrue 1-2 patients per month from the multidisciplinary pancreatic cancer clinic at the University of Michigan Comprehensive Cancer Center, therefore completing accrual to this trial in approximately 3 years.

Cohort size

Patients will be recruited as available and allocated to treatment according to the estimated toxicity rates. No formal cohort size is required; however, a total over one or more subjects, of at least 200 days of observation at Dose  $j$  must have been completed before a subject can be treated at Dose  $j+1$

## 1.0 BACKGROUND

### 1.1 Treatment of Unresectable Pancreatic Cancer

Pancreatic cancer remains mostly incurable with an overall 5-year survival of less than 5% (1). Resection is the only therapy with potential for cure, but most patients present with metastatic or unresectable disease. In the case of unresectable pancreatic cancer, the standard therapy developed in the 1980's was the combination of fluorouracil (5FU) and radiation. In the 1990's gemcitabine was shown to be superior to 5FU in patients with advanced pancreas cancer (2). Therefore, we decided to explore combining radiation therapy with gemcitabine. We were the first to show that gemcitabine is a potent radiosensitizer of human tumor cells (3) and of pancreas cancer cells (4). These initial studies and those of other investigators established that radiosensitization occurred under conditions of simultaneous dATP pool depletion and redistribution of cells into S phase (3, 5). In addition, radiosensitization depended on homologous recombination (6) and, in other studies, on mismatch repair (7). Importantly, studies in animal models showed that the combination of gemcitabine and radiation could improve the therapeutic index (8, 9).

Based on these reports, investigators began incorporating gemcitabine into the treatment of unresectable disease. Our group at the University of Michigan was the first to conduct prospective clinical trials investigating the combination of full-dose gemcitabine and radiotherapy for locally advanced unresectable cancer as well as in the adjuvant post-operative setting (10, 11). The objective of these trials was to design a regimen that would use the most effective drug, gemcitabine, at its full systemic intensity, and at the same time, take advantage of its radiosensitizing properties (4). The key conclusion from this and our subsequent preclinical (12, 13) and clinical studies adding cisplatin (14) or oxaliplatin (15) to gemcitabine in patients with locally advanced disease is that conformal radiation permits the use of full systemic doses of chemotherapy, which is important support for testing the hypotheses proposed in this clinical trial.

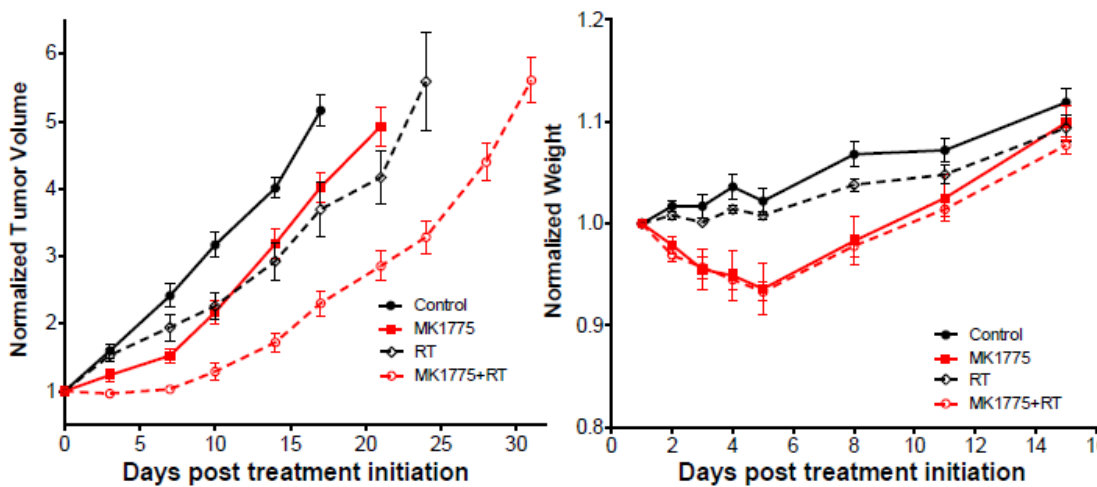
Although pancreatic cancer has a tendency to metastasize, recent evidence confirms that radiation therapy improves survival in patients who do not have gross metastatic disease. A recent phase III trial for patients with unresectable disease has shown that the combination of gemcitabine and radiation produced a statistically significant improvement in survival compared to gemcitabine alone, demonstrating that local therapy can improve survival (16). This is consistent with an autopsy study that found that 1/3 of patients who die of pancreatic cancer died due to complications of local disease progression (17).

Our data also support the hypothesis that intensification of local therapy, while maintaining full dose system therapy, can improve survival. We recently completed a radiation dose escalation study (using 1000 mg/m<sup>2</sup> of gemcitabine 2 weeks on one week off) for patients with the same eligibility criteria proposed for the current trial (18). The radiation dose level was assigned using TITE-CRM with a DLT target rate set to 0.25. Fifty patients were accrued. DLTs were observed in 11 patients: G3/4 anorexia, nausea, vomiting, and/or dehydration (7); duodenal bleed (3); duodenal perforation (1). The probability of DLT at 55 Gy (0.24) was closest to the target. The 2-year FFLP was 59% (95% CI: 32-79). Median and 2- year overall survival were 14.8 months (95% CI: 12.6-22.2) and 30% (95% CI 17-45). Twelve patients with initially unresectable disease underwent resection (10 R0, 2 R1) and survived a median of 32 months. This study showed that high dose radiotherapy with concurrent gemcitabine can be delivered safely and can produce results that are comparable to the best survival reported for patients with unresectable disease.

To summarize this section, full dose gemcitabine (alone or with oxaliplatin or cisplatin) can be administered safely with full dose radiation if conformal radiation techniques are used. Although some progress has been made by the introduction of FOLFIRINOX (19) and gemcitabine-nab paclitaxel (20) in the treatment of metastatic disease, these new combinations have not yet been demonstrated to improve survival for patients with locally advanced disease nor when combined with radiation therapy. Thus, new systemic agents, particularly those that can be combined effectively with radiation, are urgently needed.

### 1.2 The Potential of Wee1 Inhibitors for the Treatment of Pancreatic Cancer

In recent years, it has become clear that most tumor cells exhibit abnormalities in one or more aspects of cell cycle regulation (i.e., "checkpoints"), and that these abnormalities present an attractive target for therapeutic intervention. The overall concept is that defects in the cell cycle regulation systems of tumor cells may render them more vulnerable to



#### Tumor doubling time (days)

Treatment	Doubling time
Control	8 (6, 12)
MK1775	9 (5, 15)
RT	13 (10, 16)
MK1775 RT	21 (11, 26)*

MK1775 radiosensitizes MiaPaC-2 xenografts *in vivo*. Tumor bearing mice were treated with 60mg/kg BID MK1775 1h pre- and 4h post-RT (1.8Gy/fraction). Treatment was administered Mon-Fri for one cycle. Top data are normalized to the tumor volume/weight on the first day of treatment. Data are the mean  $\pm$  standard error of 10-12 tumors/5-6 mice per treatment condition. MK1775 significantly delayed tumor volume doubling in response to radiation (table).

\*P<0.05 vs RT.

drug treatments than normal cells, due to failure of checkpoints to prevent inappropriate DNA replication or mitotic entry. In particular, we have focused on Wee1 inhibitors, which can both abrogate the G2 checkpoint and inhibit homologous recombination repair. The great majority of pancreatic cancers express either mutant or non-functioning p53, and, therefore, lack a G1 checkpoint. Tumors must, therefore, depend on the G2 checkpoint to protect themselves from DNA damaging agents. Thus, a Wee1 inhibitor would be predicted to potentiate the activity of drugs, such as gemcitabine, and radiation, against tumor cells. In fact, we and others have demonstrated that a highly selective inhibitor of Wee1, AZD-1775, can sensitize to radiation and to gemcitabine (21-26). AZD-1775 is a highly selective, ATP competitive, small-molecule inhibitor of the Wee1 kinase that sensitizes tumor cells to cytotoxic agents and is being developed for the treatment of advanced solid tumors and p53 pathway deficient malignancies.

AZD-1775 alone or in combination with chemotherapy (especially gemcitabine) has been



assessed in Phase I clinical trials in over 200 patients. Preliminary analysis of mean plasma concentration profiles suggests that the overall trend of AZD-1775 exposure is approximately dose proportional at the tested dose levels. Initial studies used AZD-1775 as monotherapy. In this portion of the study, a single dose of AZD-1775 up to 1300 mg as monotherapy was generally well tolerated without DLTs. Phase I studies of a single dose of AZD-1775 in combination with cisplatin, gemcitabine, and several other agents have been completed, and a MTD has been determined in single and multiple dose combination therapy studies. A completed clinical trial of 67 patients showed that the maximum tolerated dose of AZD-1775 (given days 1 and 2) combined with Gemcitabine (1000 mg/m<sup>2</sup> on Day 1) is 175 mg, which was, therefore, set as the maximum dose for this trial. The most common AEs observed in studies of combination of AZD-1775 with chemotherapy include blood and lymphatic disorders, (i.e., thrombocytopenia, neutropenia, leukopenia, anemia, febrile neutropenia), gastrointestinal disorders (i.e., diarrhea, vomiting, nausea, abdominal pain, and constipation), and investigations (hematology and serum chemistries).

To date, AZD-1775 has been sufficiently well tolerated to permit further evaluations, including evaluations of other dosing regimens, and evaluations of regimens combining the compound with standard doses of two chemotherapeutic agents. Our proposed study will be the first that uses AZD-1775 with radiation in pancreatic cancer. (Other radiation trials are ongoing for patients with high grade glioma and cervix cancer.)

### **1.3 Summary**

Our long-term goal is to improve the survival of patients with pancreatic cancer by enhancing the efficacy of gemcitabine-radiation by adding the Wee1 inhibitor AZD-1775. In the current trial we will determine the maximum tolerated dose (which we will call the “target dose” (see statistical section below)) and toxicity profile of AZD-1775 when administered concurrently with gemcitabine-radiation. We will also carry out a correlative study to determine if Wee1 is being inhibited in patients who have received AZD-1775 at or below the target dose.

## **2.0 OBJECTIVES**

### **2.1 Primary objective**

2.1.1 To determine the target dose and toxicity profile of AZD-1775 when administered concurrently with gemcitabine and gemcitabine-radiation in patients with unresectable pancreas adenocarcinoma.

### **2.2 Secondary objectives**

2.2.1 Secondary Objective 1: To estimate the efficacy of this regimen (combined with standard systemic therapy) at the target dose, as determined by progression-free survival and overall survival at one year.

2.2.2 Secondary Objective 2: To determine if Wee1 is inhibited by AZD-1775 at or below its target dose (with gemcitabine or gemcitabine-radiation) in surrogate tissues.

## **3.0 ELIGIBILITY CRITERIA**

### **3.1 Inclusion Criteria**

3.1.1 Patients must have pathologically confirmed adenocarcinoma of the pancreas.

3.1.2 Patients will have unresectable disease, defined radiographically as >180 degrees involvement of the superior mesenteric artery or celiac trunk or SMV/portal vein impingement that cannot be surgically reconstructed, in the absence of distant metastasis.

3.1.3 Patients must have a Zubrod performance status of  $\leq 2$ .

3.1.4 Patients must have adequate organ function defined as follows: absolute neutrophil count of  $\geq 1500/\text{mm}^3$ , platelets  $\geq 100,000/\text{mm}^3$ , Hgb  $\geq 9$ , serum creatinine  $\leq 2$  mg/dl, total bilirubin  $\leq 3$ , (with relief of biliary obstruction if present (PTC tube or endobiliary stent)) and AST and Alkaline phosphatase  $< 5$  times the upper limit of normal.

3.1.5 Patients of reproductive potential must agree to use an effective contraceptive method during participation in this trial and for 6 months after the trial. Patients must not be breastfeeding.

3.1.6 Patients must be aware of the investigational nature of the therapy and provide written informed consent.

3.1.7 Patients must be at least 18 years old.

### 3.2 Exclusion Criteria

3.2.1 Other serious uncontrolled concomitant systemic disorders or psychiatric condition that would interfere with the safe delivery of protocol therapy.

3.2.2 A history of previous chemotherapy for pancreatic cancer or abdominal radiation therapy.

3.2.3 The use of any investigational agent in the month before enrollment into the study.

3.2.4 Inability to discontinue a prescription or non-prescription drugs or other products known to be metabolized by CYP3A4, or to inhibit or induce CYP3A4 prior to Day 1 of dosing and to withhold throughout the study until 2 weeks after the last dose of study medication. Medications of particular concern are the following inhibitors of CYP3A4: azole antifungals (ketoconazole itraconazole, fluconazole and voriconazole), macrolide antibiotics (erythromycin, clarithromycin), cimetidine, aprepitant, HIV protease inhibitors, nefazodone and the following inducers of CYP3A4: phenytoin, barbiturates and rifampicin. Substrates of CYP3A4 include statins (lovastatin, simvastatin, atorvastatin), midazolam, terfenadine, astemizole, and cisapride. Refer to **Appendix A** for a list of commonly used moderate and potent CYP3A4 modifiers. For other concomitant medications not on this list and known to significantly influence CYP3A4, the Investigator and Astra Zeneca will determine if it should be discontinued.

3.2.5 Pregnant women are not eligible to participate in this study.

3.2.6 Cardiac Disease ongoing or in the past 6 months (e.g. congestive heart failure, acute myocardial infarction, significant uncontrolled arrhythmias).

## 4.0 PRETREATMENT EVALUATION (Screening)

4.1 Complete history and physical examination including weight, Zubrod performance status, and review of current medications to be completed within 2 weeks of registration.

- 4.2 Diagnostic CT of the chest, abdomen and pelvis to rule out distant metastases and to establish unresectable disease should be performed within 4 weeks of registration.
- 4.3 CBC with differential, platelets, serum chemistry panel and CA 19-9 within 2 weeks of treatment start.
- 4.4 A serum pregnancy test for women of child-bearing potential
- 4.5 12 lead ECG to rule out cardiac disease.

## 5.0 REGISTRATION PROCEDURES

After informed consent is obtained and PRIOR to the initiation of protocol therapy all patients satisfying the inclusion/exclusion criteria must have eligibility confirmed by the GI SPORE Clinical Trials Team. The patient will not be considered registered and enrolled in the study until all information is confirmed by the GI SPORE Clinical Trials Data Manager.

## 6.0 TREATMENT PLAN

AZD-1775 will be given on days 1 and 2, and on days 8 and 9 of every 3-week cycle according to the protocol established by phase I trials of gemcitabine-AZD-1775 (AZD-1775 Investigator's Brochure and personal communication). Protocol therapy will consist of an initial cycle of gemcitabine-AZD-1775, followed by the combination of gemcitabine, AZD-1775, and radiation, followed by additional gemcitabine-AZD-1775 as diagrammed in the figure. The dose of AZD-1775 will be escalated according to the TITE-CRM design (see *Section 12.0 Statistical Considerations*). Radiotherapy will be delivered beginning with the second cycle of systemic therapy.

### SCHEMA

	Registration	Wk 1	Wk 2	Wk 3	Wk 4	Wk 5	Wk 6	Wk 7	Wk 8	Wk 9-11	Wk12 -14 (1 cycle)	Wk 15	Wk 15-26	to 18 months
RT*					X	X	X	X	X	Break		DLT evaluation	Optional additional cycles	F/U
Gemcitabine**	X	X		X	X			X	X		X			
AZD-1775***	X	X		X	X			X	X		X			
Skin biopsies <sup>+</sup>	X			X										

\*RT: 52.5Gy in 25 fractions (2.1Gy/fraction), using intensity modulated radiation therapy (IMRT). Radiation therapy will be administered after chemotherapy.

\*\*Gemcitabine: 800-1000 mg/m<sup>2</sup>, 30 minute, d1 and d8 of a 21-day cycle

\*\*\*AZD-1775: 125 to 175 mg (flat dosing), d1,2 and d8,9 of a 21-day cycle. The starting dose will be Level 0 (125 mg). The recommended maximum tolerated dose (MTD) when administered on days 1 and 2 with concurrent gemcitabine is estimated to be 175 mg. AZD-1775 will be administered orally 3-4 hours and 24 hours after completion of the gemcitabine infusion.

+Skin Biopsies: Two biopsies will occur on day 1 and day 2 with cycle 1 or cycle 2.

This is a dose-escalation trial to determine the maximum tolerated dose of AZD-1775 when administered with gemcitabine and radiation, in patients with unresectable pancreatic cancer. Dose-escalation will be managed by the TITE-CRM algorithm (see below), with the goal of establishing the target dose, as per the table below. Astra Zeneca has established that AZD-1775 at 175 mg QD x 2 doses with gemcitabine (1000 mg/m<sup>2</sup>) is the MTD. Therefore, our goal would be to reach 175 mg (day 1, 2 and 8,9) with full dose gemcitabine, although we would anticipate pCDC2 inhibition might be achieved at lower doses (see Sections 10 and 11 for biomarker assessment).

### Dose escalation schema

	Radiotherapy dose	Gemcitabine (mg/ m <sup>2</sup> )	AZD-1775 dose (mg)
Level -2	52.5	800	100
Level -1	52.5	1000	100
Level 0	52.5	1000	125
Level 1	52.5	1000	150
Level 2	52.5	1000	175

#### **6.1 Gemcitabine**

- 6.1.1 Gemcitabine 1000mg/m<sup>2</sup> will be infused over 30 minutes on days 1 and 8 of a 3-week treatment cycle.
- 6.1.2 A cycle of treatment may begin when ANC  $\geq$  1,000/mm<sup>3</sup>, platelets  $\geq$  75,000/mm<sup>3</sup> and all other treatment related toxicity has resolved to  $\leq$  grade 2
- 6.1.3 Dose adjustments for chemotherapy during a cycle and for subsequent cycles can be found in section 8.0.

#### **6.2 AZD-1775**

- 6.2.1 AZD-1775 will be administered, with the assistance of the University of Michigan Investigational Drug Services Pharmacy, as an oral capsule 3-4 hours and 24 hours after completion of the gemcitabine infusion.
- 6.2.2 The dose of AZD-1775 will be escalated according to the TITE-CRM design (see section 12.0)

#### **6.3 Radiation Therapy**

- 6.3.1 Treatment Planning
  - 6.3.1.1 Planning will be performed based on a helical CT obtained within 4 weeks of the initiation of radiation therapy. The CT scan will be obtained in the treatment position following administration of both oral and intravenous contrast. Helical scans will be obtained with a large (48 cm or greater) field of view, using an aperture of 3.5 mm or smaller, and a pitch of 2. The CT data will be transferred to a treatment planning system for contouring of critical structures. Active Breathing Control will be used to reduce/eliminate breathing motion. If a patient cannot tolerate ABC, a 4D CT will used to generate an internal target volume (ITV).
  - 6.3.1.2 The gross tumor volume (GTV) is defined as the gross tumor including enlarged regional lymph nodes. The clinical target volume (CTV) will be the GTV, plus a margin of 0.5 cm to account for occult invasion. An ITV will be generated as described above. The planning target volume (PTV)

will be the ITV plus 0.5 cm for daily patient set-up variation. Note that radiographically uninvolved lymph nodes will not be included in the target.

- 6.3.1.3 Treatment planning will be performed using IMRT as described previously (28), with the isocenter calculated at 100% and the 95% line encompassing 99.5% or greater of the PTV. Normal tissue tolerances will be respected as described below. Doses of > 5% above the prescribed dose will not be allowed unless they occur within the GTV.
- 6.3.1.4 Use of greater than or equal to 10 MV photons is required.
- 6.3.1.5 Normal Tissue Tolerances: The spinal cord and kidneys will be contoured into the treatment planning system, using the digital CT data. The spinal cord shall not receive greater than 45 Gy. No more than 50% of the combined functional renal volume (e.g. 20% of one kidney and 80% of the other) will receive >20 Gy. Dose to the small bowel, stomach, or colon will be limited to less than the prescription dose (i.e. no “hot spots” permitted) as described above.
- 6.3.2 Radiation Therapy
  - 6.3.2.1 Radiation therapy will typically be given during cycle 2 and 3 of chemotherapy. Radiation therapy will be given daily, five times weekly, starting on the first day of the first cycle of chemotherapy. Treatment will generally begin on a Monday or Tuesday.
  - 6.3.2.2 All fields will be treated daily.
  - 6.3.2.3 Patients will be treated in a supine position, generally with their arms up. Use of immobilization devices is required.
  - 6.3.2.4 The total dose will be 52.5 Gy in twenty-five fractions of 2.1 Gy each. This radiation dose is one dose level below the radiation dose established as the MTD for high dose radiation combined with gemcitabine.
  - 6.3.2.5 Cone Beam CT (CBCT) and/or KV imaging for patients with implanted fiducial markers will be used for image guidance and will be assessed on a daily basis. These images will be made available for review if required.

## 7.0 DRUG INFORMATION

### 7.1. Gemcitabine

- 7.1.1 Description – Gemcitabine hydrochloride (2', 2'-difluoro-2'deoxyctidine) is a deoxycytidine analog with structural and metabolic similarities to cytarabine. Gemcitabine is metabolized intracellularly by deoxynucleoside kinases to active di- and triphosphate nucleosides. The active nucleosides interfere with ribonucleotide reductase and compete with dCTP for incorporation into DNA, respectively, resulting in inhibition of DNA synthesis. Gemcitabine pharmacokinetics are linear and are described by a two compartment model. Half-life varies with age, gender, and infusion length; for a short infusion it is generally less than 70 minutes. Nearly all of an administered dose is recovered in the urine as active drug (<10%) or inactive uracil metabolite. The maximum plasma concentrations of the inactive metabolite are achieved 30 minutes after discontinuation of the infusions, and the metabolite is excreted in the urine without undergoing further biotransformation. The metabolite does not accumulate with weekly dosing, but its elimination is dependent on renal excretion, and could accumulate with decreased renal function.
- 7.1.2 Human Toxicity - Myelosuppression is the principal dose-limiting factor with gemcitabine therapy including leukopenia, thrombocytopenia and anemia.

Non-hematologic toxicities include reversible hepatic enzyme elevations, gastrointestinal toxicity (nausea, vomiting, diarrhea, and stomatitis), fever in the absence of infection, flu-like syndrome, rash, and peripheral edema. Rarely hemolytic-uremic syndrome, drug-induced pneumonitis and sepsis have been reported.

- 7.1.3 Pharmaceutical Data – Gemcitabine is supplied as a lyophilized powder in sterile vials containing 200 mg or 1 g of gemcitabine as the hydrochloride salt, with mannitol and sodium acetate. Gemcitabine is reconstituted with 0.9% sodium chloride without preservatives and used within 24 hours.
- 7.1.4 Administration – Intravenous infusion over 30 minutes (see treatment plan).
- 7.1.5 Storage and Stability – Store at controlled room temperature.
- 7.1.6 Supplier – Gemcitabine is commercially available for purchase by third party from Eli Lilly and Company.

## **7.2 AZD-1775**

- 7.2.1 Description - The chemical name for AZD-1775 is 2-allyl-1-[6-(1-hydroxy-1-methylethyl)pyridin-2-yl]-6-[[4-(4-methylpiperazin-1-yl)phenyl]amino]-1,2-dihydro-3H-pyrazolo[3,4-d]pyrimidin- 3-one. The clinical formulation of AZD-1775 contains the active ingredient in a DFC, in 10 mg, 25 mg, and 100 mg strengths (RC-DFC; PMF). AZD-1775 is the only active ingredient in the clinical formulation. AZD-1775 is a highly selective, adenosine-triphosphate (ATP) competitive, small- molecule inhibitor of Wee1 kinase. The maximum concentration of AZD-1775 is reached from 1 to 4 hours after oral dosing and the  $\frac{1}{2}$  life is 9-11 hours.
- 7.2.2 Human toxicity – In phase I trials of AZD-1775 combined with gemcitabine, the chief toxicity has been hematologic (including both neutropenia and thrombocytopenia). Fatigue and elevated transaminases (AST and ALT) have also been noted. Updated information for AZD-1775 includes new risks for complications of existing cardiac disease and potential for serious GI hemorrhage while taking AZD-1775. A rare, but serious side effect of sepsis has been reported while taking AZD-1775.
- 7.2.3 Pharmaceutical Data - AZD-1775 is a crystalline, non-hygroscopic, monohydrate of the neutral drug. It dehydrates upon heating leading to formation of a crystalline anhydrate. AZD-1775 is available for clinical trials as a roller-compacted granule of AZD-1775 and excipients (RC-DFC). The RC-DFC consists of a roller compacted granule of AZD-1775, lactose, microcrystalline cellulose, croscarmellose sodium and intragranular magnesium stearate. The granule is lubricated with a further quantity of extragranular magnesium stearate prior to encapsulation of the granule. The active potencies for the RC-DFCs are 25 mg and 100 mg in size 2 gelatin capsules.
- 7.2.4 Administration – AZD-1775 is administered as an oral capsule (see treatment plan)
- 7.2.5 Storage and Stability - The capsules are packaged in high-density polyethylene (HDPE) bottles fitted with induction seals. Clinical supplies should be stored at not more than 30°C.
- 7.2.6 Supplier – AZD-1775 is supplied by Astra Zeneca, Inc.

## 8.0 DOSAGE MODIFICATIONS

### **8.1 Dose modifications For Chemotherapy (Gemcitabine and AZD-1775) Within in a Cycle (day 8 treatment)**

- 8.1.1 Hematologic toxicity - dose adjustments of gemcitabine and AZD-1775 will be made based on the absolute neutrophil count (ANC) and platelet count taken on the day of therapy.
- 8.1.2 For ANC  $\geq 1,000$  /mm<sup>3</sup> and platelets  $\geq 75,000$ /mm<sup>3</sup>, full dose will be given.
- 8.1.3 For ANC of 500- 999/mm<sup>3</sup> and/or platelets of 50,000 to 74,999/mm<sup>3</sup>, 50% of the gemcitabine dose due will be given and AZD-1775 will be given only on day 9 (instead of both days 8 and 9).
- 8.1.4 For ANC  $< 500$ /mm<sup>3</sup> or platelets  $< 50,000$ /mm<sup>3</sup>, the treatment will be held. If this toxicity occurs during radiation therapy (cycle 2 or 3), radiation therapy will also be held, with combined modality treatment resuming upon recovery to values permitting chemotherapy. Combined modality treatment will resume (cycles 2 and 3) when ANC  $\geq 500$  and platelets  $\geq 50,000$ /mm<sup>3</sup> with gemcitabine given at 50% dose reduction. If this hematologic toxicity occurs beyond cycle 3, the dose of gemcitabine (and AZD-1775) will be dropped and not made up.
- 8.1.5 Non-hematological toxicity- Dose adjustments of chemotherapy will be made following assessment of non-hematological toxicity on the day of therapy. Chemotherapy will be held for  $\geq$  Grade 3 toxicity in any organ system. If gemcitabine and AZD-1775 are held during cycle 2 or 3, radiation therapy will also be held while appropriate physical, laboratory, radiological assessments are undertaken to define cause and direct supportive therapy. Treatment will be resumed upon recovery to toxicity  $\leq$  grade 1. If non-hematological toxicity  $\geq$  grade 3 occurs in any cycle beyond cycle 3, the chemotherapy dose will be omitted and treatment resumed when toxicity resolves to  $\leq$  grade 1 beginning a new cycle with a 25% dose reduction of gemcitabine.
- 8.1.6 If a day 8 dose of chemotherapy is delayed or omitted because of toxicity, all subsequent gemcitabine and AZD-1775 doses will be given at 75% previous dose.

### **8.2 Chemotherapy Dose Modifications for Subsequent Cycles**

- 8.2.1 A cycle of therapy may begin when ANC  $\geq 1,000$  /mm<sup>3</sup>, platelet count is  $\geq 75,000$ /mm<sup>3</sup>, and all other treatment related toxicity has resolved to  $\leq$  grade 2.
- 8.2.2 If a chemotherapy treatment is not given the day it is due for toxicity per section 7.1, all subsequent gemcitabine and AZD-1775 doses will be given at 75% of the previous dose. Once reduced for toxicity requiring delay/omission, chemotherapy doses will not be re-escalated.
- 8.2.3 If therapy is not given for treatment related toxicity and recovery allowing for treatment does not occur within four weeks of the date treatment was due, the patient is off protocol.

- 8.3 **Radiation Therapy Modifications:** Radiation will be held for up to one week when chemotherapy is held. Radiation will restart the second week even if chemotherapy does not restart. Otherwise, there will be no adjustments of the radiation therapy dose.

<b>Dose Modifications</b>			
<b>Gemcitabine (mg/m<sup>2</sup>)</b>		<b>AZD-1775 (mg)</b>	
<b>Starting Dose</b>	<b>Modified Dose</b>	<b>Starting Dose</b>	<b>Modified Dose</b>
1000	800	175	150 on Day 1, 8 125 on Day 2, 9
	650		100
	500		75
	375		50
	Off treatment		Off treatment
800	650	150	100
	500		75
	375		50
	Off treatment		50 on Day 1, 8 25 on Day 2, 9
			Off treatment
		125	100
			75
			50
			50 on Day 1, 8 25 on Day 2, 9
			Off treatment
		100	75
			50
			50 on Day 1, 8 25 on Day 2, 9
			Off treatment



## 9.0 PATIENT ASSESSMENTS AND DATA COLLECTION

### 9.1 Study Calendar

Staging CT scan will be performed within 28 days of registration. All other pre-treatment evaluations will be within 14 days of registration.

	Pre-treatment (Screening)	Cycle 1	Cycle 2-3	Cycle 4-8	Follow-up Visits <sup>9</sup>
History/Physical/confirm medications	X		X <sup>5</sup>	X	X
Performance Status	X		X <sup>5</sup>	X	X
CBC,diff,plts	X	X <sup>2</sup>	X	X	X
Serum Chemistry	X <sup>1</sup>	X <sup>5</sup>	X <sup>5</sup>	X	X
Serum Pregnancy Test	X				
CA 19-9	X			X <sup>8</sup>	X
12 lead ECG	X				
Liver function tests		X <sup>10</sup>	X <sup>10</sup>	X <sup>10</sup>	
Toxicity Notation		X <sup>2</sup>	X <sup>6</sup>	X <sup>5</sup>	X
CT chest/abdomen/pelvis	X			X <sup>8</sup>	X
Gemcitabine		X <sup>3</sup>	X	X	
AZD-1775		X <sup>4</sup>	X	X	
Radiation Therapy			X <sup>7</sup>		
Assess for adverse events	X	X	X	X	X
Skin Biopsies		X <sup>11</sup>	X <sup>11</sup>		

<sup>1</sup> Serum chemistry panel will include albumin, alkaline phosphatase, ALT, AST, glucose, total bilirubin, creatinine and electrolytes.

<sup>2</sup> Weekly on the day chemotherapy is given

<sup>3</sup> Gemcitabine by 30 minute IV infusion given days 1 and 8 (section 6.1)

<sup>4</sup> AZD-1775 orally days 1, 2 and 8, 9 (section 6.2)

<sup>5</sup> On day 1 of each chemotherapy cycle, except on day 1 of cycle 1 when pre-treatment values can be used.

<sup>6</sup> Weekly during radiation therapy

<sup>7</sup> Daily treatment M-F for 5 weeks (section 6.3)

<sup>8</sup> Prior to cycle 4 and after last cycle.

<sup>9</sup> Approximately every 3 months for 18 months, then every 4 months +/- 2 months for another 18 months.

<sup>10</sup> Obtained every other week (alone or as part of chemistry panel (see footnote 1)), and includes bilirubin, AST, ALT, and alkaline phosphatase

<sup>11</sup> Skin biopsies before and after AZD-1775 administration, day one and day two of cycle 1 or cycle 2.

### 9.2 Follow up Schedule

With completion of treatment, patients will be seen in follow-up approximately every 3 months for 18 months, then every 4 months +/- 2 months for another 18 months.

### **9.3 Toxicity Assessment**

Toxicity will be assessed using the Common Terminology Criteria for Adverse Events (CTCAE) version 3.0. Toxicity will be assessed on each chemotherapy visit, weekly during radiation therapy and at each follow-up visit. Dose-limiting hematologic and non-hematologic toxicities will be defined differently, and will be based on events occurring up to 2 months after the completion of chemoradiation (approximately 105 days after the first day of treatment). In order to be declared a dose-limiting toxicity, an adverse experience must be at least possibly related (i.e. definitely, probably, or possibly) to study therapy

### **9.4 Dose Limiting Toxicity (DLT)**

- 9.4.1 Grade 4-5 hematological toxicity with the exception of Grade 4 anemia, Grade 4 leukopenia, Grade 4 neutropenia lasting for <7 days and Grade 4 thrombocytopenia lasting for <4 days, EXCEPT if a platelet transfusion is required (in other words, Grade 4 thrombocytopenia that requires a platelet transfusions considered a DLT).;
- 9.4.2 Grade 3 or Grade 4 neutropenia with fever >38.5°C and/or infection requiring antibiotic or anti-fungal treatment
- 9.4.3 Grade ≥3 non-hematological toxicity, except:
  - 9.4.3.1 nausea, vomiting, or diarrhea that in the opinion of the investigator occurs in the setting of inadequate compliance with supportive care measures and lasts for less than 48 hours
  - 9.4.3.2 hyperbilirubinemia/cholangitis secondary to biliary obstruction
  - 9.4.3.3 any grade of alopecia;
  - 9.4.3.4 inadequately treated hypersensitivity reactions
  - 9.4.3.5 clinically non-significant, treatable or reversible lab abnormalities including such as glucose, uric acid, etc.
- 9.4.4 Missed doses: if a patient misses 50% of the scheduled dose of AZD-1775 during the observation period (4 cycles/ 8 doses), it will be considered a DLT (except if due to poor patient compliance in the absence of treatment-related toxicity). For example, if a patient is assigned 125 mg x 8 doses, then without missing or reducing dose patient would take 1000 mg. If the patient was able to receive < 500 mg, it would be considered a DLT.
- 9.4.5 A delay of >2 weeks in the scheduled administration of radiation, gemcitabine, and AZD-1775 due to drug-related toxicities which do not qualify as a DLT per the guidelines above (except if the delay is due to a cause unrelated to the disease or treatment under study (e.g. family emergency or auto accident) will be scored as a DLT.

### **9.5 Definition of Outcome Measures**

- 9.5.1 Progression Free Survival: Time from date of registration to the date of documented progressive disease, other disease related therapy or death.
- 9.5.2 Response Rate: Objective response assessed from the CT scan obtained prior to cycle 4 and again at completion of study treatment.
- 9.5.3 Freedom from Local Progression: Time from date of registration to the date of documented local progressive disease.
- 9.5.4 Overall Survival: Time from date of registration to date of death or last follow up.

### **9.6 Criteria for Removal from Study**

- 9.6.1 Evidence of progressive disease.
- 9.6.2 Treatment delay of > 4 weeks.
- 9.6.3 Patient refusal to continue therapy.
- 9.6.4 Toxicity that despite protocol specified delay and dose reduction requires treatment cessation.

## 10.0 STUDY MONITORING

### 10.1 Adverse Event Reporting Guidelines

10.1.1 Adverse events monitoring and reporting is a routine part of every clinical trial and is done to ensure the safety of subjects enrolled in the studies as well as those who will enroll in future studies using similar agents. Data on adverse events will be collected from the time of informed consent through 30 days after the last study treatment. Any serious adverse event that occurs more than 30 days after the last treatment and is considered related to the study treatment must also be reported. Serious Adverse Events (SAEs) will continue to be followed until:

- Resolution of the symptoms or signs that constitute the serious adverse event return to baseline;
- There is satisfactory explanation other than the study treatment for the changes observed; or
- Death.

The investigator is responsible for the detection, documentation, grading, and assignment of attribution of events meeting the criteria and definition of an AE or SAE. The definitions of AEs and SAEs are given below. It is the responsibility of the principal investigator to ensure that all staff involved in the trial is familiar with the content of this section.

Any medical condition or laboratory abnormality with an onset date before initial study treatment is considered to be pre-existing in nature. Any known pre-existing conditions that are ongoing at time of study entry should be considered medical history.

All events meeting the criteria and definition of an AE or SAE, as defined in Section 10.1.2, occurring from the time of informed consent through 30 days following the last study treatment must be recorded as an adverse event in the patient's source documents and on the CRF regardless of frequency, severity (grade) or assessed relationship to the study treatment.

In addition to new events, any increase in the frequency or severity (i.e., toxicity grade) of a pre-existing condition that occurs after the patient begins study treatment is also considered an adverse event.

#### 10.1.2 Definitions

##### Adverse Event

An adverse event (AE) is any untoward medical occurrence in a patient receiving study treatment and which does not necessarily have a causal relationship with this treatment. An AE can be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of

an experimental intervention, whether or not related to the intervention.

- Diagnostic and therapeutic non-invasive and invasive (i.e., surgical) procedures will not be reported as adverse events. However, the medical condition for which the procedure was performed must be reported if it meets the definition of an adverse event unless it is a pre-existing (prior to protocol treatment) condition.
- Abnormal laboratory values or test results constitute adverse events if they induce clinical signs or symptoms or require therapy. They are to be captured under the signs, symptoms or diagnoses associated with them.
- All AEs will be categorized according to system organ class for annual reporting to the FDA. Only grade 3 and above AEs, which are noted in the medical record to be related to the study treatment (RT + gemcitabine +AZD-1775) will be reported to the IRB. SAE's will be reported per 10.1.4.
- During the course of an adverse event, severity and/or causality and/or seriousness may change. For CRF documentation this adverse event represents one entity from onset to resolution and the worst of the observed categories shall be attributed
- When event reoccurs after it disappeared, it should be handled as a new AE. However, AEs that occur intermittently can be recorded as one AE

#### Serious Adverse Event

An adverse event is considered "serious" if, in the view of the investigator it results in any of the following outcomes:

- Death  
If death results from (progression of) the disease, the disease should be reported as event (SAE) itself.
- A life-threatening adverse event  
An adverse even is considered 'life-threatening' if, in the view of the investigator its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization for  $\geq$  24 hours.

- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Important medical event  
Any event that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient and may require medical or surgical intervention to prevent one of the outcomes listed in this definition of “Serious Adverse Event”. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home; convulsions that do not result in inpatient hospitalization or the development of drug dependency or drug abuse.
- Any events or hospitalizations that are unequivocally due to progression of disease (with the exception of death) should not be reported as a SAE. The causality of SAEs (i.e., their relationship to study treatment) will be assessed by the investigators per section 8.2.2

Previously planned (prior to signing the informed consent form) surgeries should not be reported as SAEs unless the underlying medical condition has worsened during the course of the study. Preplanned hospitalizations or procedures for preexisting conditions that are already recorded in the patient’s medical history at the time of study enrollment should not be considered SAEs. Hospitalization or prolongation of hospitalization without a precipitating clinical AE (for example, for the administration of study therapy or other protocol-required procedure) should not be considered SAEs. However, if the preexisting condition worsened during the course of the study, it should be reported as an SAE. Hospitalization or emergency room visits secondary to expected cancer morbidity, such as admission for palliative care or pain management will not constitute a SAE.

#### Expected Adverse Events

An adverse event (AE) is considered “expected” if:

- For approved and marketed drugs or devices, those adverse events are described in the approved Package Insert (Label).
- For investigational new drugs or devices, those adverse events are described in the FDA Investigator’s Brochure.
- In clinical research studies, information on expected adverse events is also summarized in the protocol and in the consent document. See section 9.1 for the list of expected adverse events related to the drug under study.

### Unexpected Adverse Event

An adverse event (AE) is considered “unexpected” if it is not described in the Package Insert, Investigator’s Brochure, in published medical literature, in the protocol, or in the informed consent document.

### 10.1.3 Adverse Event Characteristics

#### CTCAE Term

(AE description) and grade: The descriptions and grading scales found in the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 3.0. (We recognize that the current CTCAE list version is 4.0. However, all prior studies with AZD-1775 have been conducted using version 3.0, and this scoring will greatly facilitate comparison to prior studies.)

#### Attribution of the AE

The investigator or co-investigator is responsible for assignment of attribution.

Definite – The AE is clearly related to the study treatment.

Probable – The AE is likely related to the study treatment.

Possible – The AE may be related to the study treatment.

Unlikely – The AE is doubtfully related to the study treatment.

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

### 10.1.4 Serious Adverse Event Reporting Guidelines

The Principal Investigator must be notified within 2 business days of study team’s knowledge of any event meeting the criteria and definition of a serious adverse event related to study treatment occurring during the study or within 30 days of the last administration of the study related treatment.

Only adverse events deemed serious and related will be reported to the IRB within 7 days of awareness of the event. All other events will be noted in the patient’s medical record and reported to the IRB according to IRB guidelines.

#### Serious Adverse Event (SAE) Reporting to Astra-Zeneca and FDA:

Investigator shall forward to Astra\_Zeneca all serious adverse experiences as detailed in the contract with the sponsor.

The Study Team will coordinate with the Michigan Institute for Clinical and Health Research (MICHR) IND/IDE Investigator Assistance Program

(MIAP) office for the reporting of any and all IND safety reports to the FDA as per the requirements outlined in 21 CFR 312.32. SAEs

meeting the requirements for expedited reporting (unexpected and related) will be submitted to the FDA within 7 calendar days.

#### 10.1.5 Routine Reporting

Adverse events will no longer be reported if the patient has another pancreas-directed therapy

#### 10.1.6 Reporting of Unanticipated Problems

Upon becoming aware of any incident, experience, or outcome (not related to an adverse event) that may represent an unanticipated problem, the investigator should assess whether the incident, experience, or outcome represents an unanticipated problem. The incident, experience, or outcome is considered unanticipated if it meets all of the following criteria:

1. Unexpected (in terms of nature, severity, or frequency);
2. Related or possibly related to participation in the research; and
3. Suggests that the research places subjects or others at a greater risk of harm than was previously known or recognized.

If the investigator determines that the incident, experience, or outcome represents an unanticipated problem, the investigator must report it to the IRB within 7 calendar days of the study team becoming aware of the problem if the problem is serious, and within 14 calendar days if the problem is non-serious.

### **10.1 Data and Safety Monitoring**

- 10.2.1 This trial will be monitored in accordance with the NCI approved University of Michigan Comprehensive Cancer Center Data and Safety Monitoring Plan.

The study specific Data and Safety Monitoring Committee (DSMC), consisting of the protocol investigators, data manager or designee and other members of the study team involved with the conduct of the trial, will meet monthly or more frequently depending on the activity of the protocol to provide continuous review of the data and patient safety. The discussion will include matters related to the safety of study participants (SAE/UaP reporting), validity and integrity of the data, enrollment rate relative to expectations, characteristics of participants, retention of participants, adherence to the protocol (potential or real protocol deviations) and data completeness.

At the regular DSMC meetings, the protocol specific Data and Safety Monitoring Report form will be completed. The report will be signed by the Principal Investigator or by one of the co-investigators.

Data and Safety Monitoring Reports will be submitted to the University of Michigan Comprehensive Cancer Center Data and Safety Monitoring Board (DSMB) on a monthly basis for independent review.

## 11 DETERMINATION OF THE EFFECT OF AZD-1775 ON WEE1 ACTIVITY

### 11.2 Biopsies for Surrogate Markers

During the first or second cycle of treatment, patients will undergo 2 biopsies: the first will occur 3 h after treatment with gemcitabine (but before AZD-1775) on day 1 of cycle 1 or 2, and the second will occur approximately 3-5 hours after AZD-1775 administration on day 2 of cycle 1 or 2. We will obtain 3mm punch biopsies of the skin to evaluate replicating cells in hair follicles. Patients who refuse skin biopsies are still eligible for treatment.

### 11.3 Biomarkers

Utilizing immunohistochemistry, we will assess Wee1 signaling in response to AZD-1775 in combination with gemcitabine. Using a punch biopsy of the skin to evaluate replicating cells in hair follicles, we hypothesize that AZD-1775 plus gemcitabine will reduce Cdk1 (Y15) phosphorylation relative to gemcitabine alone, confirming Wee1 inhibition. To confirm Wee1 pathway inhibition, we will assess the mitotic marker pHistoneH3, and anticipate that it will be increased in response to AZD-1775 plus gemcitabine relative to gemcitabine alone. To confirm the presence of DNA damage in response to AZD-1775 we will evaluate  $\gamma$ H2AX and pChk1 (S345) (29). We expect that AZD-1775 plus gemcitabine will lead to an induction of these surrogate markers of DNA damage. Other potential biomarkers of AZD-1775 activity include Caspase-3 cleavage and Rad51 foci. Eight genes (CLSPN, CCNE1, MCM10, MYB, CCNE2, FBX05, EGR1, and HIST1H2BD) have been identified as potential candidates for PD markers by microarray analysis by other investigators and will also be evaluated.

## 12 STATISTICAL CONSIDERATIONS

This is a phase I dose-escalation trial to determine the maximum tolerated dose of AZD-1775 when administered with gemcitabine  $\pm$  radiation, in patients with unresectable pancreatic cancer. Dose-escalation will be managed by the TITE-CRM algorithm, with the goal of establishing the target dose (see below) (14, 30-32). Section 12.1 describes the TITE-CRM design in detail and provides operating characteristics under several simulation scenarios. Section 12.2 describes the proposed analysis addressing each of the study aims.

In mid-2017, after the enrollment of 24 patients, the target rate of DLT was increased from 25% to 30%. A rate of 30% is within the normal range of target rates, particularly in this patient population receiving Chemo-RT in addition to the investigational agent. Data from all previously treated patients will continue to be utilized in estimating posterior probabilities of DLT at each dose level.

### 12.1 Trial Design

12.1.1 **Number of Patients:** 36 subjects evaluable for toxicity will be accrued to this trial. Based on recent recruitment, it is expected that 15 subjects can be accrued to this trial per year. The trial will be run until either 36 patients evaluable for toxicity have been enrolled



OR the estimated probability of toxicity at the lowest dose level exceeds 35%, in which case the trial would be stopped early.

- 12.1.2 **Dose Assignment:** The first patient will be treated at level 0. Each subsequent patient's dose level will be assigned using the TITE-CRM algorithm. When a new subject is enrolled, the probability of toxicity at each dose is estimated, based on the toxicity data accrued up to that time, using a one-parameter logistic dose-toxicity model described below. The subject is assigned to the highest dose with estimated probability of toxicity closest to the target rate of 30% but not exceeding 35%, subject to two dose-escalation restrictions: (1) the dose of subject  $i$  cannot be more than one level greater than the dose of subject  $i-1$ ; (2) a total, over one or more subjects, of at least 200 days of observation at Dose  $j$  must have been completed before a subject can be treated at Dose  $j+1$ .
- 12.1.3 **Target Dose:** The target dose will be that which produces DLT at a frequency of 0.30. That is, the goal of the trial is to treat patients at the dose associated with DLT in 30% of patients.
- 12.1.4 **Initial Estimates of Probability of Toxicity:** Before any subjects are treated, the estimated probability of toxicity at each dose level is given in the following table. The sensitivity of operating characteristics of the trial (number of toxicities, quality of estimation of target dose) to these estimates is assessed by means of Monte Carlo simulations.

	Prior Values	Rescaled Dose ( $d_{rs}$ )	True Probabilities Used in Simulation		
			Scenario 1	Scenario 2	Scenario 3
Dose Level	$P_d$		$p_{d1}$	$p_{d2}$	$p_{d3}$
-2	0.05	-5.94	0.05	0.05	0.15
-1	0.10	-5.20	0.10	0.10	<b>0.25</b>
0	0.15	-4.73	0.15	0.15	0.40
1	0.20	-4.39	0.20	<b>0.25</b>	0.55
2	0.25	-4.10	<b>0.25</b>	0.40	0.65

*Dose ( $d$ ), initial estimate of probability of toxicity for dose  $d$  ( $d_{rs}$ ), rescaled dose values, and true probabilities of toxicities used in Monte Carlo simulations ( $p_{d1}$ - $p_{d3}$ ) of TITE-CRM trials.*

- 12.1.5 **Dose-toxicity function:** A continuous, monotonically increasing function relating treatment dose to the probability of toxicity is employed to allow subjects' experience at any dose to contribute to the estimation of the probability of toxicity at all doses. The function is an approximation to the true state of nature; the trial estimates are robust against misspecification of this function. This trial will use a logistic dose-toxicity model:

$$p(\text{DLT}|d)=\exp(3+\alpha \cdot d_{rs})/(1+\exp(3+\alpha \cdot d_{rs})),$$

where  $\alpha$ , the dose-toxicity parameter, is to be estimated, and  $t_d$  is the rescaled dose, calculated from the initial estimate of probability of toxicity ( $pd$ ), assuming  $\alpha=1$ .

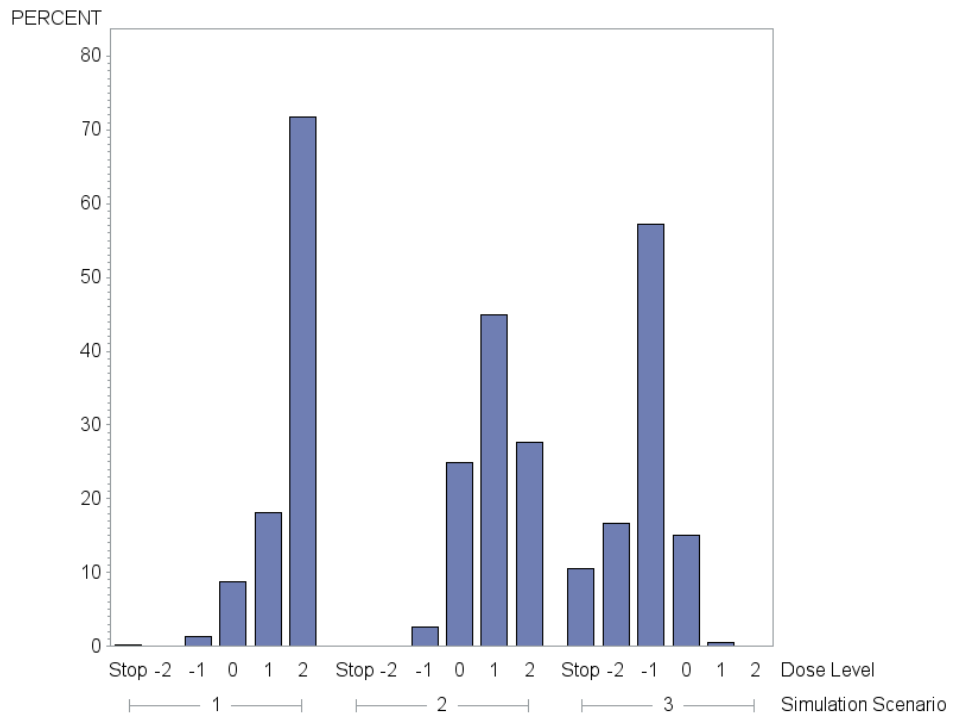
12.1.6 **Estimation of  $p(DLT|d)$ :** The prior distribution of the dose-toxicity parameter,  $\alpha$ , is Gaussian, with mean 1 and standard deviation 0.3. At any time, the probability of toxicity at each dose is estimated by calculating the expected value of alpha, given the prior and the trial data up to that time, and entering the expected value of alpha into the logistic dose-toxicity function. At a given point of time, the datum of subject  $i$  is represented by the triple  $(d_i, y_i, w_i)$ , where  $d_i$  denotes the dose the subject received,  $y_i$  the response (0=no DLT, 1=DLT) and  $w_i$  the weight (per section 12.1.7 below) derived from the portion of the approximately 105-day observation period the subject has completed at the given time, or, if the subject has experienced DLT, 1. The expectation of  $\alpha$  can be calculated using a straightforward univariate numerical integration, and is implemented in a SAS code that has been used in a number of trials.

12.1.7 **Observation Period:** Observation for DLT (as defined in Section 9.4) will begin on the first day of treatment, and continue until an assessment at a follow-up visit following (~day 21 of) cycle 4 (prior to optional cycle 5 and beyond, approximately day 105). Subjects who are rendered unevaluable for DLT during this period for reasons unrelated to toxicity of treatment (e.g., disease progression) will not be counted towards the accrual goal, but will be fractionally counted in the estimation of the probability of toxicity. The first cycle will be weighted more than later cycles because proportionally more DLT's are expected during cycle 1. The cumulative weight assigned to a subject who completes each cycle without DLT is given below. Subjects who experience DLT have weight 1.0 regardless of when the DLT occurred.

Day 0	Cycle 1	Cycle 2	Cycle 3	Cycle 4
0	.5	.7	0.9	1.0.

12.1.8 **Operating Characteristics:** Operating characteristics, estimated by means of Monte Carlo simulation, are presented below. The primary goal of the simulations is to check the design robustness against the misspecification of the dose-conditional probability of toxicity. For this presentation, 1000 trials were simulated for each of three sets of characteristics (Section 12.1.5), where the true state of nature was as specified in the protocol ( $pd_1$ ), more toxic than assumed ( $pd_2$ ), and much more toxic than assumed, with a rapid increase in toxicity near the target rate ( $pd_3$ ). Metrics presented include the distribution of selected target doses (a measure of estimation efficacy), the number of observed toxicities (a measure of risk) and the numbers of subjects treated at each dose (indicating the number of subjects treated at potentially therapeutic doses). In each of the 3 simulations, the correct dose is the most frequently selected dose. When the priors are correct the highest dose level

is selected in greater than 70% of trials. When the priors are far off, as in the case of Simulation 3, the correct dose level is still selected in more than half of all trials. Simulation 3 also represents the potentially riskiest state of nature, but even in this case the median number of DLT's is 11 (31% of the total 36 patients). In all three simulations, the average number of subjects treated at the target dose or within 1 level, is over 25, allowing for Phase IIa estimates of efficacy. It has been previously demonstrated [30] that, in general, the TITE-CRM design is not significantly riskier (in terms of number of toxicities/subjects treated) than the 'traditional' design, while better estimating the target dose, and treating a greater number of subjects at potentially therapeutic doses.



Simulation	Cumulative Number of DLTs Per Trial				
	10 <sup>th</sup> Percentile	25 <sup>th</sup> Percentile	Median	75 <sup>th</sup> Percentile	90 <sup>th</sup> Percentile
1	5	6	7	8	10
2	7	8	9	10	11
3	7	9	11	12	13

Simulation	Average Number of Patients Treated at Each Dose Level				
	-2	-1	0	1	2
1	0	2	8	9	17
2	0	2	10	12	12
3	7	13	10	3	0

*Operating characteristics of example TITE-CRM trial: Upper plot shows the percent of simulated trials selecting each dose level as the target dose. In the table showing the average number of patients treated at each dose level, the*

*numbers may not sum to 36 due to rounding or to the fact that some trials stop early and thus enroll fewer than 36 patients.*

## 12.2 Data Analysis plan

**Primary Objective:** To determine the target dose and toxicity profile of AZD-1775 when administered concurrently with gemcitabine and gemcitabine-radiation in patients with unresectable pancreas adenocarcinoma.

At the end of the trial, once all evaluable subjects have completed observation,  $p(\text{DLT}|d)$  will be calculated for each dose using the tite-CRM method (one parameter logistic regression model with prior distribution on the parameter alpha). The final estimated target dose will be the dose  $d$  with estimated  $p(\text{DLT}|d)$  closest to the target rate but not exceeding 35%. To further characterize toxicity as a function of dose, we will use maximum likelihood to fit a two parameter logistic regression model to the data from this trial. This more flexible model typically provides estimates of toxicity very close to the tite-CRM estimates at and near the target dose, but better estimates toxicity at doses further from the selected target dose. The numbers of subjects treated at each dose and the numbers experiencing DLT will be tabulated. Toxicities at each dose level will be tabulated, categorized by grade and by whether they are not, possibly, probably or definitely related to treatment. Toxicities not considered dose limiting may also be summarized in this manner and modeled as a function of dose level.

**Secondary Objective 1:** To estimate the efficacy of this regimen (combined with standard systemic therapy) at the target dose, as determined by progression-free survival, overall survival, freedom from local progression and response rates.

Overall survival (OS), progression-free survival (PFS) and freedom from local progression (FFLP) will be summarized by Kaplan-Meier curves and characterized by descriptive statistics such as median OS and PFS times. For OS, subjects alive at the time of last FU will be censored at that time. Similarly, for PFS and FFLP, subjects alive and without progression will be censored at the last date on which they were assessed for progression. Cox proportional hazards regression models will be used to estimate OS, PFS and FFLP as a function of dose and possibly other covariates such as age or inhibition of Wee1. Response will be assessed per RECIST at 4 months. The number and proportion of patients with progressive disease, stable disease, partial and complete response will be calculated for each dose level and overall. Logistic regression models will be used to model probability of any response as a function of dose level. Exploratory analyses will be conducted to assess for any relation between Wee-1 inhibition and efficacy outcomes.

Although efficacy is only a secondary aim of this trial and it is not formally powered for efficacy, we nevertheless show that the proposed design has reasonable power to make early efficacy assessments. Specifically, we calculate the power of the design to compare the observed overall survival at 1 year to that in historical control patients. The estimated overall survival at 1 year in Murphy et al was 46%. In each of the 3 simulation scenarios above, there were at least 25 patients treated at the target dose  $\pm 1$ . Based on these numbers and a 1-sided .15 level chi-square test, the proposed design has at least 77% power to detect an improvement

of 1 year survival to 65%. In addition to aggregating survival data for patients treated at the target dose with those treated one dose level above or below, we will look for a dose response relationship using a logistic or Cox regression model. Based on this fitted model, we will also compare the model-estimated outcome at the target dose level to the historical control data.

Secondary Objective 2: To determine if Wee1 is inhibited by AZD-1775 at or below its target dose (with gemcitabine or gemcitabine-radiation) in surrogate tissues.

During the first or second cycle of treatment, patients will undergo 2 biopsies: 3 h after treatment with gemcitabine (but before AZD-1775), and on day 2 approximately 3-5 hours after AZD-1775. WEE1 signaling will be assessed using IHC to measure phosphorylation of various markers including Cdk1 (Y15). Inhibition will be quantified as the within subject change in the above markers between the two biopsy timepoints. Descriptive statistics of inhibition across subjects (for each marker) will be calculated and reported by dose level. Regression models of inhibition as a function of AZD-1775 dose will be fit to obtain smooth and efficient estimates of mean inhibition at a given dose level. In addition to mean inhibition, the proportion of patients with inhibition greater than 0 (i.e. positive change) or another fixed level of interest will be estimated via logistic regression models. Since AZD-1775 dose is of primary interest in this aim, patients in dose levels -2 and -1 may be lumped together since they receive the same AZD-1775 dose and only their dose of gemcitabine differs.

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Appendix A New PDF of drug interactions is added to the pdf version-  
word version is not available from Astra Zeneca.