THE UNIVERSITY OF TEXAS
MD Anderson Cancer Center

# Tissue Pharmacokinetics of Intraoperative Gemcitabine in Adenocarcinoma of the Pancreas after Preoperative Chemoradiation Therapy

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## List of Abbreviations

| | |
|---|---|
| AST/SGOT | Aspartate aminotransferase |
| ALT/SGPT | Alanine aminotransferase |
| CBC | Complete blood count |
| CDA | Cytidine deaminase |
| Cl | Clearance |
| dCDA | Deoxycytidine deaminase (gemcitabine inactivating enzyme) |
| dCK | Deoxycytidine kinase (gemcitabine activating enzyme) |
| dCTP | Deoxycytidine triphosphate |
| dFdC | Gemcitabine (2',2'-difluorodeoxycytidine) |
| dFdCDP | Difluorodeoxycytidine diphosphate |
| dFdCTP | Difluorodeoxycytidine triphosphate |
| dFdU | Difluorodeoxyuridine (a gemcitabine metabolite) |
| DNA | Deoxyribonucleic acid |
| DVT | Deep vein thrombosis |
| ELISA | Enzyme-linked immunosorbent assay |
| EMR | Electronic medical record |
| FDR | Fixed dose rate |
| GI | Gastrointestinal |
| GV | Gauri Varadhachary, MD |
| hENT-1 | Human equilibrative nucleoside transporter 1 |
| Hh | Hedgehog |
| HPLC/MS | High performance liquid chromotography-mass spectrometry |
| HUS | Hemolytic-uremic syndrome |
| HW | Huamin Wang |
| IHC | Immunohistochemistry |
| JF | Jason Fleming, MD |
| LC/MS/MS | Liquid chromatography-mass spectometry |
| µmol | Micromole |
| $m^2$ | Meters squared |
| Mets | metastases |
| mg | Milligram |
| min | Minute |
| mL | Milliliter |
| mm | Millimeter |
| mol | Mole |
| mmol | Millimole |

| | |
|---|---|
| MT | Mark Truty, MD |
| OR | Operating room |
| PBMC | Peripheral blood mononuclear cells |
| PC | Pancreatic cancer |
| PCR | Polymerase chain reaction |
| pK | Pharmacokinetics |
| RT-PCR | Reverse transcription polymerase chain reaction |
| SMA | Superior mesenteric artery |
| SMV | Superior mesenteric vein |
| SMPV | Superior mesenteric portal vein |
| SNPs | Single-nucleotide polymorphisms |
| TUNEL | Terminal deoxynucleotidyl transferase dUTP nick end labeling |
| UTI | Urinary tract infection |
| VTE | Venous thromboembolism |
| WHO | World Health Organization |
| WP | William Plunkett, PhD |

## 1.0 Objectives

### 1.1 Primary Objective:

- To quantifiably assess intratumoral gemcitabine levels in human pancreatic cancer tissue after a single intraoperative infusion in patients who have completed a course of preoperative chemotherapy and radiation therapy.

### 1.2 Secondary Objectives:

- To measure intra-DNA gemcitabine (dFdC) levels using a novel assay LC/MS/MS, developed by Eli Lily, available through Advion BioServices.

- To assess and validate previously described markers that may be predictive of gemcitabine sensitivity or resistance - examine the expression of molecules involved in gemcitabine metabolism (*CDA, dCK, DCTD)*, and transport (*hENT1, hCNT1, hCNT2, hCNT3)* in the resected primary tumors.

- To measure the impact of microvessel density and the molecular expression level of the Hh signaling pathway on gemcitabine delivery and DNA incorporation. Blood vessels in resected tumors will be detected by IHC using antibodies to CD31 or vWF. The mRNA and protein expression of *SHH, Gli* and *SMO* will be measured by RT-PCR and IHC method, respectively. These markers will be correlated to the gemcitabine level.

- To correlate intratumoral gemcitabine levels and its tumoricidal activity with Ki67 index and intratumoral apoptosis.

## 2.0 Background and Rationale

**2.1** Gemcitabine is a nucleoside analogue that exhibits modest antitumor activity in numerous solid tumors, including pancreatic adenocarcinoma, and is currently the first-line agent for this malignancy.[1] There is no current data that demonstrates that this drug in fact penetrates tumor tissue despite the increasing use of this agent in the operable and locally advanced pancreatic cancer setting. **This is even more critical a question for previously radiated tumors**. Emerging evidence suggests that resistance to gemcitabine in pancreatic cancer may be related to several critical factors: a desmoplastic stroma with poor vascular permeability that inhibits drug delivery into cancerous tissue, and variable expression of key proteins involved in transport, activation, metabolism, and elimination of this agent.[2-6]

**2.2** Previous clinical Phase I/II pharmacokinetic studies in pancreatic cancer have only measured gemcitabine levels within peripheral blood.[7-10] In these studies the efficacy of gemcitabine has been correlated with concentrations accumulated in peripheral blood mononuclear cells (PBMC), which are related to plasma concentrations of the drug. Classically these PBMC's are used to quantify the levels of active gemcitabine and its metabolites in tumor tissue, however they are only a surrogate matrix. Unfortunately there is no data to suggest that gemcitabine or its active intracellular metabolite, gemcitabine triphosphate (dFdCTP), is present to any

significant level in human pancreatic cancer tissue despite this agent now being the standard-of-care**. In patients with locally advanced pancreatic cancer, it is not unusual in clinical practice to give additional gemcitabine after completion of radiation without any knowledge that the drug actually enters radiated tissue.**

**2.3** Recent data suggests that the de novo resistance may be related to poor penetration of the therapeutic agent into the pancreatic tissue. By way of example, many patients display resistance to even the standard first-line therapy – Gem. (2) Some of the factors that have been described as contributing to resistance to Gem (dFdC) include: (i) Hypoperfusion (ii) Desmoplastic stroma (iii) Factors involved in its uptake, metabolism, and elimination - deoxycytidine kinase deficiency (dCK – Gem activating enzyme), higher deoxycytidine deaminase expression (dCDA - Gem inactivating enzyme) and hENT1 (transporter) deficiency in tumor cells. (13-16). Further detail into the action of these key regulatory genes and their role in gemcitabine activity within *in vivo* cancerous tissue warrants further investigation in a clinical trial setting.

**2.4** We currently have a novel pharmacokinetic human clinical trial (2010-0371) in which patients undergoing curative resection of pancreatic adenocarcinoma receive an intraoperative single infusion of gemcitabine. The purpose of this clinical and translational science is to specifically assess and quantify drug incorporation within *in vivo* cancerous tissues in order to develop a novel and effective model for Phase 0/1 trials in the neoadjuvant setting to enable us to ask mechanistic questions pertaining to drug delivery and discovery in pancreatic cancer. Gemcitabine is a safe, well-studied, and commonly used drug in this tumor model – and currently 7 patients have completed the study (2010-0371) without any drug related toxicity or concerns. We plan to extend this approach to additional patients who have received preoperative chemoradiation and undergoing tumor resection.

**2.5** Previous preclinical, biochemical, and clinical investigations have strongly suggested that the rate of gemcitabine triphosphate accumulation is saturated at 10 to 20 mol/L of gemcitabine in plasma or medium. This concentration of gemcitabine is achieved in plasma when gemcitabine is infused at a dose rate of 10 mg/m2/min (Fixed dose rate, FDR). A phase II trial by Tempero et al compared FDR to standard infusion and pharmacokinetic analyses demonstrated a two-fold increase in intracellular gemcitabine triphosphate concentration in the FDR arm (P =.046). The selected dose for this trial will be lower than the full dose of 1000mg/m2/min since in practice after completion of RT, the dose is often decreased and most patients are given up to 750mg/m2. After safety is established with a lower dose of 500mg/m2, we will increase the dose to 750mg/m2.

**2.6** Quantification of gemcitabine incorporation into human DNA by liquid chromatography-mass spectometry (LC/MS/MS) is a novel assay which is available through Advion BioServices.. These methods will normalize the amount of dFdC released relative to the native (DNA) nucleoside dG and simultaneously quantify the signal of both analytes by LC/MS/MS by extensive detuning of the signal for dG. This method represents an improved surrogate for quantitating gemcitabine target engagement and results will be compared to intracellular gemcitabine (metabolite) levels in circulating PBMCs and PC tumor tissue and will be correlated to the markers in the secondary objectives.

**2.7** With preliminary data demonstrating the feasibility and promise of this approach, we will address important issues including impact of therapeutic intervention, including pre-operative radiation on drug penetration (potentially change the treatment paradigm for borderline and locally advanced pancreatic cancer).

## 3.0 Patient Eligibility

### 3.1 Inclusion Criteria:

**3.1.1** Cytologic or histologic proof of adenocarcinoma of the pancreas is required. Patients with Islet cell tumors are not eligible.

**3.1.2** Patients do not have known metastases.

**3.1.3** Patients must have potentially resectable or borderline resectable pancreatic cancer and have agreed to undergo surgical resection at MD Anderson Cancer Center if operable. They will have undergone staging (physical examination, chest x-ray, contrast enhanced CT or MRI (if CT contraindicated) and/or angiogram) to determine resectability.

**3.1.4** Patients have completed radiation and chemotherapy with either capecitabine or gemcitabine as a radiosensitizing agent as part of their preoperative therapy. Previous systemic chemotherapy alone is not allowed. Preoperative therapy will be completed at least 4 weeks prior to surgery.

**3.1.5** Patients with Karnofsky performance status > 70 are eligible. (Appendix 1)

**3.1.6** There will be no upper age restriction. Patients less than 18 years of age are excluded from the protocol because adenocarcinoma of the pancreas is rarely seen in the pediatric population.

**3.1.7** Adequate renal and bone marrow function:

- Leukocytes >= 3,000/uL
- Absolute neutrophil count >= 1,500/uL
- Platelets >= 100,000/Ul
- Serum creatinine <= 2.0 mg/dL
- Creatinine clearance >= 60 ml/min (calculated by the Cockroft-Gault equation)

**3.1.8** Adequate Hepatic function (endoscopic or percutaneous drainage as needed)

- Total bilirubin < = 3 X institutional upper limits of normal (ULN)
- AST (SGOT)/ALT (SGPT) <= 5 X institutional ULN

**3.1.9** Patients must have no fever or evidence of infection or other coexisting medical condition that would preclude administration of gemcitabine. Patients with uncontrolled congestive heart failure, unstable angina and myocardial infarction within 3 months will be excluded

**3.1.10** Patient is not pregnant. Women of childbearing potential (i.e., women who are pre-menopausal or not surgically sterile) must use acceptable contraceptive methods (abstinence, intrauterine device [IUD], oral contraceptive or double barrier device) and refrain from breast-feeding, as specified in the informed consent.

**3.1.11** Patients must sign a study-specific consent form.

### 3.2 Exclusion criteria:

**3.2.1** Major cardiovascular or pulmonary comorbidity that precludes use of general anesthesia (NYHA [New York Heart Association] Class III and IV) (Appendix 7)

**3.2.2** Identification of metastatic disease.

**3.2.3** Patients with a known hypersensitivity to Gemcitabine.

**3.2.4** Pregnant women

**3.2.5** Inability to comply with study and/or follow-up procedures.

**3.2.6** Patients < 18 years of age.

## 4.0 Gemcitabine

### 4.1 Pharmacology

**4.1.1** Gemcitabine is commercially available from Eli Lilly and Company, Indianapolis, Indiana. **Drug supply for this study will be purchased with Institutional Research Grant funds through Investigational Pharmacy** Services. Investigational Pharmacy Services will dispense the drug. Patients will not be charged for the cost of the drug.

**4.1.2** Gemcitabine HCl is 2-deoxy-2, 2-difluorocytidine monohydrochloride (beta isomer) nucleoside analogue.

**4.1.3** The main cytotoxic effect of gemcitabine involves the competitive incorporation of gemcitabine triphosphate into replicating DNA leading to masked chain termination, which inhibits DNA synthesis and leads to cell death.[17-19] Gemcitabine exhibits cell phase specificity, primarily killing cells undergoing DNA synthesis (S-phase) and also blocking the progression of cells through the G1/S-phase boundary. Gemcitabine is a pro-drug that must be phosphorylated to its active metabolites, difluorodeoxycytidine diphosphate (dFdCDP) and difluorodeoxycytidine triphosphate (dFdCTP) nucleosides, intracellularly by nucleoside kinases to have biological activity. The cytotoxic effect of gemcitabine is attributed to a combination of two actions of the

diphosphate and the triphosphate nucleosides, which leads to inhibition of DNA synthesis. First, gemcitabine diphosphate inhibits ribonucleotide reductase, which is responsible for catalyzing the reactions that generate the deoxynucleoside triphosphates for DNA synthesis. Inhibition of this enzyme by the diphosphate nucleoside causes a reduction in the concentrations of deoxynucleotides, including dCTP. Second, gemcitabine triphosphate competes with dCTP for incorporation into DNA. Gemcitabine diphosphate (dFdCDP) induces a self-potentiating mechanism by inhibiting ribonucleotide reductase, thereby reducing the amount of intracellular dCTP. After the gemcitabine nucleotide is incorporated into DNA, only one additional nucleotide is added to the growing DNA strands. After this addition, there is inhibition of further DNA synthesis. DNA polymerase epsilon is unable to remove the gemcitabine nucleotide and repair the growing DNA strands (masked chain termination) after which the DNA synthesis is inhibited and cell death occurs.

## 4.2 Pharmacokinetics

- Gemcitabine pharmacokinetics are linear and are described by a 2-compartment model. Population pharmacokinetic analyses of combined single-and multiple-dose studies showed that the volume of distribution of gemcitabine was significantly influenced by duration of infusion and gender. Volume of distribution is 50 $L/m^2$ – 370 $L/m^2$ depending on length of infusion and protein binding is negligible.

- Clearance is dependent on age and gender and is primarily renal. Cl is 75.7 – 92.2 $L/h/m^2$ (men) and 57 – 69.4 $L/h/m^2$ (women). In elderly populations, Cl is 55.1 $L/h/m^2$ (men) and 41.5 $L/h/m^2$ (women). Half-life of drug is 42 (short infusion) – 638 (long infusion) minutes depending on length of infusion time.

## 4.3 Gemcitabine Preparation, Administration, Storage, and Disposal:

**4.3.1** Gemcitabine is a white to off-white lyophilized powder available in sterile single-use vials containing 200mg or 1000mg gemcitabine.

**4.3.2** Gemcitabine will be prepared according to the institutional standard. Caution should be exercised in handling and preparing gemcitabine solutions and the use of gloves is recommended. Drug vials will be reconstituted by the institutional inpatient operating room pharmacy with 0.9% sodium chloride without preservatives to a maximum concentration of 38mg/ml due to solubility considerations. A predetermined amount of drug (see gemcitabine administration plan for dosing) will be prepared for administration as a continuous infusion. Once the drug has been reconstituted, it should be stored at room temperature and used within 24 hours.

**4.3.3** **Gemcitabine will be administered by the anesthesiologist**. Gemcitabine will be infused at a fixed dose rate of 10$mg/m^2$/min. Previous preclinical, biochemical, and clinical investigations have strongly suggested that the rate of gemcitabine triphosphate accumulation is saturated at 10 to 20 mol/L of

gemcitabine in plasma or medium.[9, 12, 20] This concentration of gemcitabine is achieved in plasma when gemcitabine is infused at a dose rate of 10mg/$m^2$/min. Although at present the data in the target tumor tissue are lacking for such comparisons, the data in surrogate PBMC's strongly suggest the benefit of this pharmacologically based gemcitabine infusion rate and serves as a dose rate to provide plasma concentrations (approximately 20 mol/L) that are sufficient to maximize the rate of gemcitabine triphosphate accumulation within cancerous tissue.

**4.3.4** Stored at controlled room temperature (20-25°C), gemcitabine is stable for 24 hours. Solutions of reconstituted gemcitabine will not be refrigerated. No incompatibilities have been observed with infusion bottles or polyvinyl chloride bags and administration sets. All unused portions will be handled and disposed of in a manner consistent with other anti-cancer drugs according to institutional policies.

## 4.4 Gemcitabine Precautions and Toxicities:

**4.4.1** Gemcitabine is contraindicated in those with a known hypersensitivity to the drug.

**4.4.3** Previous studies using a similar intraoperative approach in central nervous system cancers, found no complications related to single-dose infusion of Gemcitabine.[21] Earlier Phase I/II trials at this institution found little toxicity with initial single-dose therapy. Our current clinical trial using intraopeartive Gemcitabine is accruing steadily and no major complications related to gemcitabine use have been reported (IRB 2010-0371).

See Appendix 2 for a full list of Gemcitabine related side effects as mentioned in the package insert.

## 5.0 Gemcitabine Administration Plan

This study is limited to patients who have been determined previously to have potentially resectable or more likely, borderline resectable pancreatic cancer, according to current surgical practice at MD Anderson. The preoperative and postoperative tests/evaluations referenced in this study are standard of care for this patient population and would be performed regardless of whether a patient enrolled in this study or not. The tests/evaluations are described in this study to demonstrate good clinical practices and because the results will be used to document eligibility for this study or as part of postoperative safety monitoring.

## 5.1 Pre-surgical Evaluation:

**5.1.1** Patients will have the routine laboratory and radiologic studies required for their surgery.

## 5.2 Intraoperative Gemcitabine Administration (Appendix 3):

**5.2.1** Surgery will be planned within 4 weeks of enrollment.

**5.2.2** There will be no increase in the anesthesia or operative time, nor will the operative procedure be increased in complexity as a result of this protocol. Standard operating room procedures and intravenous fluid and medication administration will be performed for pancreatic resection per anesthesiology and surgical teams.

**5.2.3** Diagnostic laparoscopy will be performed in all patients. Patients will be excluded from the protocol if metastases are found during laparoscopy.

**5.2.4** Following the initial laparoscopic exploration patients will proceed to formal laparotomy to allow for inspection of all peritoneal surfaces and viscera including liver. If on laparotomy, the tumor is found to be locally advanced and unresectable; the patient will be excluded from the protocol.

**5.2.5** In the absence of identified metastases, full tumor mobilization and surgical resection will be performed according to surgical oncology principles.

**5.2.6** Gemcitabine will be administered intravenously as a dose of 500mg/$m^2$ at a fixed dose rate of 10mg/$m^2$/min for the first 5 patients (to validate hematologic safety). Next 15 subsequent patients will receive 750mg/$m^2$ at a fixed dose rate of 10mg/$m^2$/min. The drug infusion will be started 50-75 minutes prior to complete gross tumor removal (timing dependent on dose) in order to have drug administration complete at tumor removal ( +/- 10 minutes). Appendix 3 describes the intraoperative time points. Any deviations in the time window allowed for chemotherapy administration will not be considered a protocol deviation.

**5.2.7** Blood will be drawn for pK and correlative studies at various time points during the procedure as described in Appendix 3.

**5.2.8** Following tumor removal, specimen will be taken to Pathology lab for subsequent analysis and preparation for correlative studies.

## 5.3 Standard Anesthesia Management Plan

Drugs commonly used during pancreatic cancer surgery are listed below. These drugs have no reported interactions with gemcitabine. In the event any drugs not on this list are to be given to the patient, the anesthesiologist will confirm that there are no reported interactions with gemcitabine and the drug prior to administration.

- Amiodarone
- Antiemetics (both seratonin inhibitors and dopamine antagonists)
- Bupivicaine
- Calcium chloride
- Cisatracurium
- Dopamine

- Ephedrine
- Etomidate
- Fentanyl
- H2 blockers (cimetidine, ranitidine, famotidine, nizatidine)
- Hydromorphone
- Lidocaine
- Metoprolol
- Phenylephrine
- Propofol
- Rocuronium
- Ropivicaine
- Sufentanil

## 6.0 Safety Monitoring

### 6.1 Postoperative Patient Evaluation:

**6.1.1** Examinations, vital signs, laboratory tests and any additional imaging will follow routine practice guidelines post pancreaticoduodenectomy.

Patients will be admitted post-operatively and followed closely as per standard of care post-pancreatic resection. This includes blood work, with CBC at least every other day. We will terminate the study if we observe >33% of the first 10 patients with grade 3 or 4 adverse events that are thought to be related to gemcitabine except for transient (lasting <= 48 hours) grade 3-4 leukopenia and/or neutropenia.

Patients will be closely monitored but we do not anticipate any significant toxicity after one dose/infusion of gemcitabine. The drug labeling and literature reports of gemcitabine toxicity are on patients who have received multiple infusions of gemcitabine (at least one–two full cycles which is 3-6 infusions of gemcitabine and at a higher weekly dose).

Patients will be followed for adverse events for 30 days post surgery. Patients will be followed daily while in the hospital. Additional follow-up will occur at the time of routine clinic visits, or by a phone call to the patient at least once weekly.

**6.1.2** Our institution and study investigators have extensive experience in preoperative therapy and pancreatic resection for adenocarcinoma of the pancreas.[22, 23] Appendix 4 lists incidence of all major and minor complications. Any significant deviations from expected morbidity or mortality will be reviewed by the Study Chair, Co-Chairs, and Dr. James Yao prior to proceeding with enrollment of additional patients to the protocol. Dr. Yao will not enroll patients on this study. His role is to provide additional safety monitoring.

**6.1.3** Meetings will be held after a cohort of 3 patients who have undergone resection and received intraoperative gemcitabine to monitor safety and review

correlative data. These will be attended by the Chair, Co-Chairs, and the research team (research nurses, data manager, and laboratory research personnel). Data will be compiled in a comprehensive research overview table and shared at each meeting. In addition, laboratory values and adverse events (AE) will be documented for all patients who receive gemcitabine.

Weekly registration reports will be generated to monitor patient accruals. Routine data quality reports will be generated to assess missing data and inconsistencies. Accrual rates will be monitored periodically throughout the study period and potential problems will be brought to the attention of the study team for discussion and action.

**6.1.4** Only the designated clinical investigators will obtain informed consent/authorization. The research nurse will confirm eligibility and compliance of the consent process before entering patients into the CORe system.

## 7.0 Processing of Samples and Outcome Measurements

### 7.1 Blood Samples:

**7.1.1** Blood samples will be collected, processed, and analyzed in a single institutional laboratory (Dr. William Plunkett, T6.3916). Patients will not be charged for any costs for processing of blood and tissue samples related to this specific protocol.

**7.1.2** From each patient studied, 5-10 mL of blood will be taken into sterile heparinized tubes containing tetrahydrouridine (in order to inhibit degradation of active gemcitabine) at predetermined timepoints depending on the total gemcitabine dose. Plasma will be obtained by centrifugation of a portion of blood samples at 4°C and stored at −20°C until required for analysis of circulating plasma levels of gemcitabine and its metabolite. Gemcitabine and dFdU in tetrahydrouridine-treated human plasma are stable for at least half a year at −20°C.[24] The Ficoll-gradient method will be used for isolation of mononuclear blood cells. These cells will be immediately frozen in liquid nitrogen and subsequently stored -80°C until analysis of intracellular gemcitabine.

### 7.2 Tissue Samples:

**7.2.1** Tissue obtained will be from freshly excised tumor (superficial and deep), adjacent normal pancreas, and normal duodenal mucosa as shown in Appendix 5. These tissue specimens will be immediately frozen in liquid nitrogen and subsequently stored at -80°C until analysis (Dr. William Plunkett's laboratory, T6.3916). Previous studies have found that such samples remain stable for analysis for 2–3 years under these storage conditions.[25] The remaining resected cancer specimen will undergo full pathological evaluation.

### 7.3 Correlative Studies on Blood and Tissue Samples:

**7.3.1** The quantification of serum, PBMC, and cancer tissue levels of gemcitabine from frozen samples will be assessed using standardized techniques in high performance liquid chromatography-mass spectrometry (HPLC/MS).[24, 25]

**7.3.2** Blood vessels in resected tumors will be detected by immunohistochemistry (IHC) using antibodies to CD31 or vWF. The mRNA and protein expression of *SHH, Gli* and *SMO* will be measured by RT-PCR and IHC method, respectively. The range of levels in patients with PC will be established. We will also correlate these markers to the gemcitabine levels measured in the cancerous tissue.

**7.3.3.** The expression level of genes involved in gemcitabine metabolism (*CDA, dCK, DCTD)* and transport (*hENT1, hCNT1, hCNT2, hCNT3)* in the resected primary tumors will be examined. The mRNA level will be measured using real time PCR and protein expression by IHC. Gene expression level will be correlated to the gemcitabine measurements. Additionally, we will measure markers of proliferation and apoptosis by p21 and Ki67 IHC and fluorescent TUNEL analysis.

**7.3.4** Gemcitabine incorporation in DNA will be quantified by a new proprietary LC/MS/MS method developed by Eli Lilly (available through Advion BioServices) and performed by Advion BioServices. DNA extracted from the tissue samples will be sent to Advion (in batches of 50 samples including normal tissue).

**Advion BioServices, Inc.:**

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## 8.0 Statistical Considerations

### 8.1 Methodology and Sample Size:

This pilot protocol is a non-randomized, non-blinded regimen.

We plan to have 20 patients in the study (i.e., those who will undergo potentially curative resection and receive gemcitabine infusion).

To reach the planned 20 patients, up to 40 patients may need to be enrolled (consented) at a rate of 2-3 patients/month. Those patients who do not undergo curative PC surgery and receive gemcitabine infusion will be deemed ineligible and excluded from the analysis. The reason for enrolling up to double the number of patients is that since a significant portion of these patients may have borderline resectable disease, only 50-60% of the enrolled patients' cancers may be finally resected (data based on our large retrospective study).

**If we observe >= 2 patients in the 500mg/m$^2$ lower** dose group (5 patients) with grade 3 or 4 adverse events that ***are thought to be related*** to gemcitabine except for transient (lasting <= 48 hours) grade 3-4 leukopenia and/or neutropenia, the **study will be halted and will be amended to lower the dose/rate of infusion**.

**We will terminate** the study if **we observe >33% of the first 10 patients** with grade 3 or 4 adverse **events *that are thought to be related to gemcitabine*** except for transient (lasting <= 48 hours) grade 3-4 leukopenia and/or neutropenia.

An institutional departmental staff statistician will approve and oversee all statistical matters regarding current protocol and any subsequent analyses and publications.

The primary objective is a feasibility objective and is not amenable to statistical analysis. Analyses for the secondary objectives are exploratory in nature and will be conducted via the Spearman's Rank Correlation Coefficient.

## 9.0 Data and Protocol Management

After obtaining informed consent**,** patients will be registered on the MDACC computerized Clinical Oncology Research (CORe) System. Appendix 6 shows assignment of individual protocol responsibilities.

## 10.0 Guidelines for Monitoring Clinical Trials

**10.1** Reporting of adverse events will be according to MD Anderson Guidelines for AE Reporting.

**10.2** **This study will utilize the Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0 for toxicity grading and adverse event reporting**. A copy of the CTCAE version 4.0 can be downloaded from the CTEP home page (http://ctep.cancer.gov).

## 11.0 Confidentiality

This study will be conducted in accordance with all applicable privacy laws, rules and regulations. The Principal Investigator will take steps to guard against any loss of confidentiality. Only the Principal Investigator, Study Co-Chairs, and the authorized research team will have access to the identifiable information from this study.

Identifiers (such as name and medical record number) will be collected but will be replaced by study numbers in the analytic file. The key linking to these numbers will be retained in a secure computer file. Access to this file will be limited to the Principal Investigator, Study Co-Chairs, and the authorized research team. All computer files are password-protected and stored on institution computers behind the institution firewall to further ensure database security and all records are kept confidential. Any reports or publications resulting from this study will not include any personal identifiers.

Procedures for patients who consent to the optional banking of any left-over blood and/or tissue samples for future research: IRB approval will be obtained prior to the use of the banked samples for any research not described in this protocol. The samples will be given a code number. No identifying information will be directly linked to the samples. Only the researcher team in charge of the bank will have access to the code numbers and be able to link the samples to the subject. If a patient withdraws his/her consent for banking of samples, the banked samples will be destroyed. However, if any samples were previously released for research prior to the withdrawal of consent, the samples will not be able to be destroyed.

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**Appendix 1**

**Karnofsky Performance Status Scale:**

| Description | Status Score |
|---|---|
| Normal, no evidence of disease | 100 |
| Able to perform normal activity with only minor symptoms | 90 |
| Normal activity with effort, some symptoms | 80 |
| Able to care for self but unable to do normal activities | 70 |
| Requires occasional assistance, cares for most needs | 60 |
| Requires considerable assistance | 50 |
| Disabled, requires special assistance | 40 |
| Severely disabled | 30 |
| Very sick, requires active supportive treatment | 20 |
| Moribund | 10 |

**Appendix 2**

**Reported Gemcitabine Toxicity (all of the listed toxicities were reported with prolonged treatment, high doses, and in multiple-cycle studies, thus not anticipated to any significant degree in our single-low-dose protocol):**

- Hematologic: Myelosuppression is the dose-limiting toxicity with gemcitabine, but <1% of patients discontinued therapy for anemia, leukopenia, or thrombocytopenia. Red blood cell transfusions were required by 19% of patients. The incidence of sepsis was less than 1%. Petechiae or mild blood loss (hemorrhage), from any cause, were reported in 16% of patients; less than 1% of patients required platelet transfusions.

- Gastrointestinal: Nausea and vomiting were commonly reported (69%) but were usually mild to moderate. Severe nausea and vomiting (WHO grade 3/4) occurred in <15% of patients with prolonged infusions and doses. Diarrhea was reported by 19% of patients, and stomatitis by 11% of patients with prolonged infusions and doses.

- Hepatic: Gemcitabine was associated with mild transient elevations of serum transaminases in approximately two-thirds of patients, but there was no evidence of increasing hepatic toxicity with either longer duration of exposure to gemcitabine or with greater total cumulative dose.

- Renal: Mild proteinuria and hematuria were commonly reported. Clinical findings consistent with the hemolytic-uremic syndrome (HUS) were reported in 0.25% of patients receiving gemcitabine in clinical trials. Four patients developed HUS on prolonged gemcitabine therapy, two immediately post-therapy. Renal failure may not be reversible, even with discontinuation of therapy, and dialysis may be required.

- Fever: The overall incidence of fever was 41%. This is in contrast to the incidence of infection (16%) and indicates that gemcitabine may cause fever in the absence of clinical infection. Fever was frequently associated with other flu-like symptoms and was usually mild and clinically manageable.

- Rash: Rash was reported in 30% of patients. The rash was typically a macular or finely granular maculopapular pruritic eruption of mild-to-moderate severity, involving the trunk and extremities with prolonged infusions and doses. Pruritus was reported in 13% of patients.

- Pulmonary: Dyspnea was reported in 23% of patients, severe dyspnea in 3%. Dyspnea may be due to underlying disease, such as lung cancer (40% of study population) or pulmonary manifestations of other malignancies. Dyspnea was occasionally accompanied by bronchospasm (<2% of patients). Rare reports of parenchymal lung toxicity consistent with drug-induced pneumonitis have been associated with the use of gemcitabine.

- Edema: Peripheral edema (13%), and generalized edema (<1%) were reported. Less than 1% of patients discontinued due to edema.

- Flu-like Symptoms: "Flu syndrome" was reported for 19% of patients. Individual symptoms of fever, asthenia, anorexia, headache, cough, chills, and myalgia, were

commonly reported. Fever and asthenia were also reported frequently as isolated symptoms. Insomnia, rhinitis, sweating, and malaise were reported infrequently. Less than 1% of patients discontinued due to flu-like symptoms.

- Infection: Infections were reported for 16% of patients with prolonged infusions and doses. Sepsis was rarely reported (<1%).

- Alopecia: Hair loss, usually minimal, was reported by 15% of patients.

- Neurotoxicity: There was a 10% incidence of mild parasthesias and a <1% rate of severe parasthesias.

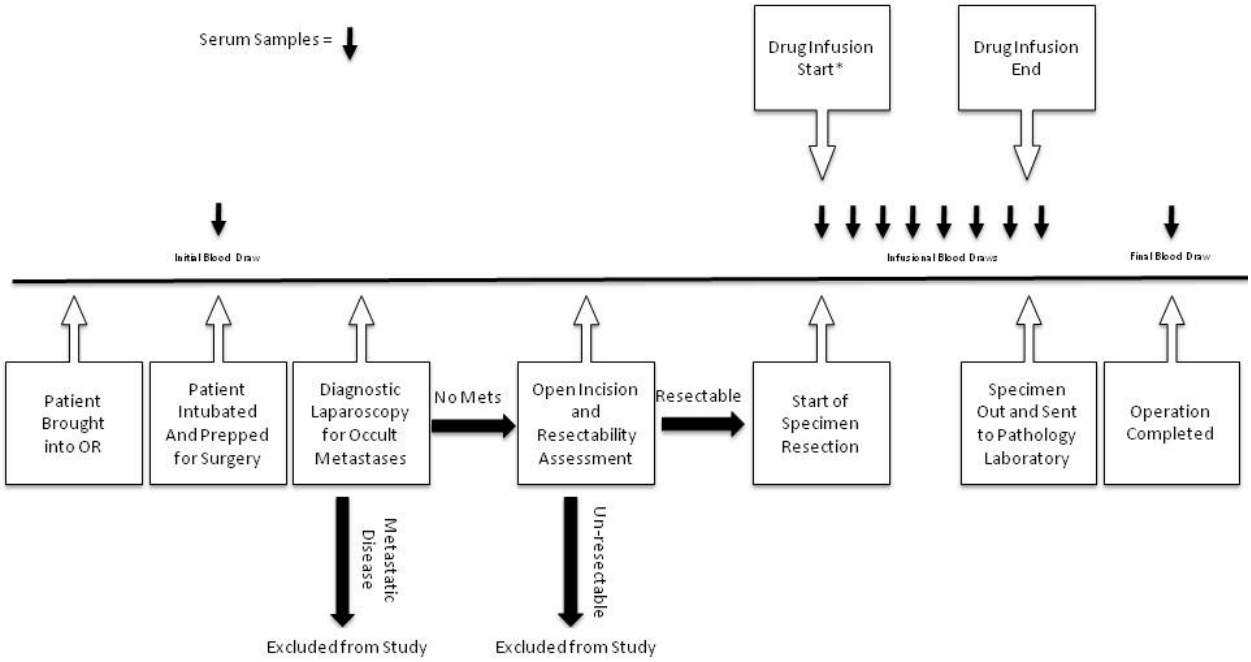
- Extravasations: Injection-site-related events were reported for 4% of patients. There were no reports of injection-site necrosis. Gemcitabine is not a vesicant.

- Allergic: Bronchospasm was reported for less than 2% of patients. Anaphylactoid reaction has been reported rarely. Gemcitabine should not be administered to patients with a known hypersensitivity to this drug.

- Cardiovascular: 2% of patients discontinued therapy with gemcitabine due to cardiovascular events such as myocardial infarction, cerebrovascular accident, arrhythmia, and hypertension. Many of these patients had a prior history of cardiovascular disease.

## Appendix 3

### Intraoperative Schema:




### Timing of Blood Draws:

| Drug Dose | Timing of Blood Draws (Gemcitabine infused at 10mg/m$^2$/min) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 500mg/m$^2$ (Initial 5 patients) | Pre-incision | 0 min | 10 min | 20 min | 30 min | 40 min | 50 min (at completion) | Approximately 70 min* |
| 750 mg/m$^2$ (Subsequent patients) | Pre-incision | 0 min | 15 min | 30 min | 45 min | 60 min | 75 min (at completion) | Approximately 95 min* |

*Last time point 20 minutes after Gemcitabine infusion completed.

**Appendix 4**

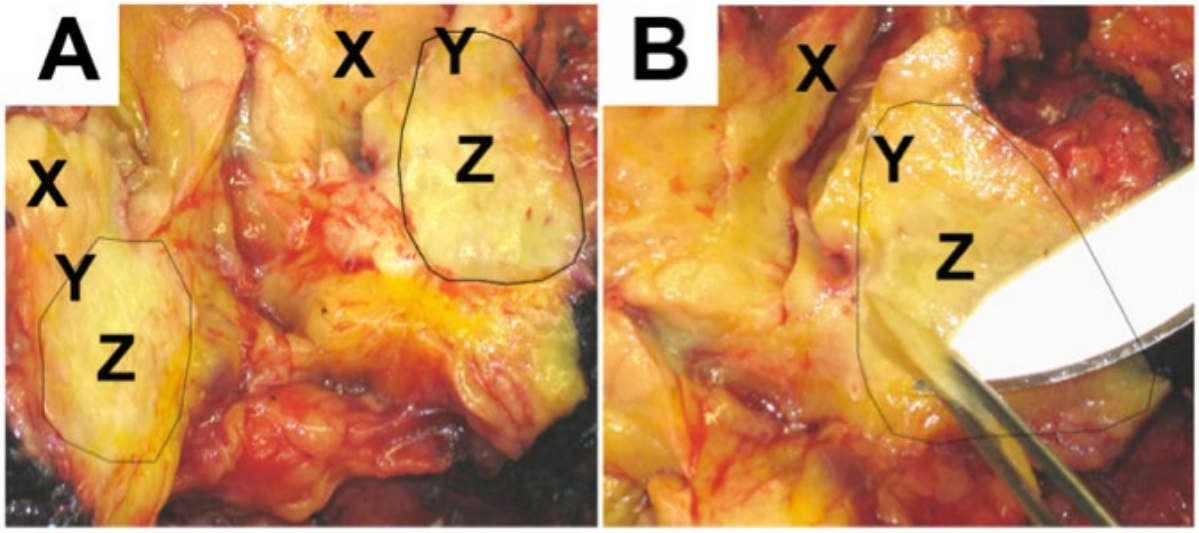
**Anticipated Perioperative Complications (as determined by previous institutional experience in pancreatic resection):**

| Complication | Anticipated Incidence |
|---|---|
| **Mortality** | **1-2%** |
| **All Major Complications** | **30%** |
| Anastomotic Leak/Fistula | 10-20% |
| Abscess/Fluid Collection | 10-15% |
| Pneumonia | 5-10% |
| DVT | 1-10% |
| GI Bleed | 1-5% |
| Intra-abdominal Bleed | 1-2% |
| | |
| VTE | 1-2% |
| Sepsis | 1-2% |
| Myocardial Infarction | 1-2% |
| Reoperation | 1-2% |
| **All Minor Complications** | **70%** |
| Atelectasis | 40-60% |
| Pleural Effusion | 20-40% |
| Delayed Gastric Emptying | 15-20% |
| Wound Infection | 5-10% |
| Cardiac Arrhythmia | 5-10% |
| Ileus | 5-10% |
| UTI | 1-10% |
| Cellulitis | 1-5% |
| Line Infection | 1-5% |
| Chyle Leak | 1-2% |

**<u>Appendix 5 Photographs</u> Depicting a Gross Pathologic Examination of the Pancreaticoduodenectomy Specimen:**

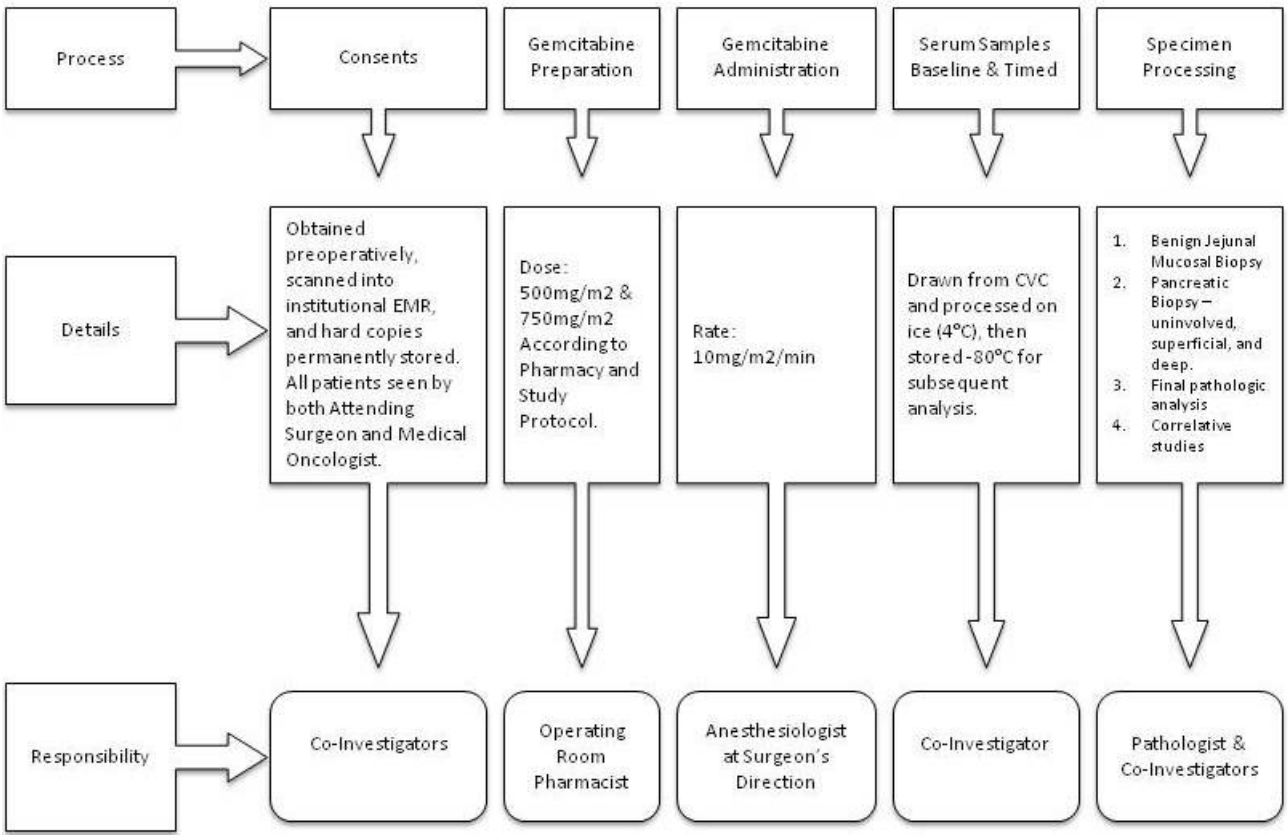
- Figure A: X = Adjacent Normal Tissue; Y = Superficial Carcinoma; Z = Deep Carcinoma.

- Figure B: X = Adjacent Normal Tissue; Y = Superficial Carcinoma; Z = Deep Carcinoma.



## Appendix 6

### Assignment of Protocol Responsibilities



## Appendix 7

**New York Heart Association (NYHA) Functional Classification:**

| NYHA Class | Symptoms |
|---|---|
| I | No symptoms and no limitation in ordinary physical activity, e.g. shortness of breath when walking, climbing stairs etc. |
| II | Mild symptoms (mild shortness of breath and/or angina) and slight limitation during ordinary activity. |
| III | Marked limitation in activity due to symptoms, even during less-than-ordinary activity, e.g. walking short distances (20–100 m).<br>Comfortable only at rest. |
| IV | Severe limitations. Experiences symptoms even while **at rest**. Mostly bedbound patients. |