A Phase II Study of Preoperative Systemic Chemotherapy (modified FOLFIRINOX) followed by Radiation therapy for Patients with High Risk Resectable and Borderline Resectable Adenocarcinoma of the Pancreas

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1.0 OBJECTIVES

1.1 Primary Objective

To assess resectability rate in patients treated with preoperative modified FOLFIRINOX (5-fluorouracil, oxaliplatin and irinotecan) chemotherapy followed by gemcitabine -based chemoradiation therapy for borderline resectable adenocarcinoma of the pancreas.

1.2 Secondary Objectives

To assess margin resection rate (R0 vs. R1) in these patients
To assess disease free survival (DFS) and overall survival (OS)
To assess patterns of local and distant failure
To evaluate correlative studies including DPC4 staining and circulating tumor cells (CTC) and CT based imaging biomarker

2.0 BACKGROUND AND RATIONALE

2.1 Borderline resectable pancreatic cancer is an important, emerging entity with improvement in imaging techniques. Rigorous criteria that define "borderline resectable" pancreatic cancer allow for appropriate patient accrual to clinical trials examining the utility of chemotherapy and/or chemoradiation delivered prior to pancreatic resection for exocrine cancer. Radiographic criteria for borderline resectable pancreatic cancer are defined as follows (patient may have one or more of the following criteria):

- Tumor abutment of ≤180degrees (≤ 50% of the vessel circumference) of the superior mesenteric artery (SMA),
- Short segment abutment or encasement (≥ 50% of the vessel circumference) of the common hepatic artery (typically at the gastroduodenal artery origin),
- Superior mesenteric vein (SMV) narrowing or impingement or segmental venous occlusion with a patent vessel above and below suitable for reconstruction.

Additionally patients with a high CA19-9 (>500), indeterminate liver or peritoneal nodules, and large peripancreatic lymph nodes are considered high risk for surgery even in the presence of operable disease and fit the criteria for borderline resectability.

2.2 The rationale for pursuing preoperative chemotherapy and chemoradiation treatment for patients with borderline resectable pancreatic cancer includes: (1) potential for down staging in order to maximize the chances for a margin negative (R0) resection, (2) treating micrometastatic disease early, (3) giving "adjuvant" therapy in a "neoadjuvant" setting when it is better tolerated, and (4) using this approach to gauge the aggressiveness of the cancer

and thereby select patients for surgery who have the greatest likelihood of a favorable postoperative outcome especially given the morbid nature of the surgery. Data also suggests that preoperative chemoradiation may decrease the incidence of pancreaticojejunal anastomotic fistula, the most common complication following pancreaticoduodenectomy or distal pancreatectomy.

2.3 Patients with borderline resectable pancreatic cancer are poor candidates for upfront surgery because they are at a very high risk for margin positive resection with initial surgery. Patients with margin positive resection do poorly with a life expectancy between 8-12 months, which is no different from patients with locally advanced pancreatic cancer. Therefore, we favor a treatment schema that incorporates preoperative (neoadjuvant) therapy with systemic chemotherapy and chemoradiation and this notion has been embraced by several institutions and high volume pancreatic cancer centers. Patients whose tumors show radiographic stability or regression often accompanied by an improvement in serum tumor markers are candidates for surgery. Patients are typically treated with preoperative therapy for 4-6 months prior to consideration of surgery.

2.4 We have successfully completed five preoperative trials at M.D. Anderson Cancer Center (Appendix A, Tables 8and 9) for resectable pancreatic cancer. The current treatment schema for borderline resectable cancer builds upon our previous work with preoperative chemoradiation for resectable disease (protocols ID98-020, ID01-341). To summarize, we have enrolled a total of 176 patients on the last two gem-based protocols for resectable pancreatic cancer and have significant experience with this regimen. The most common cause of patients not going to surgery was metastatic disease and only 1 out of 176 patients had local progression that precluded surgery. Also the overall median survival of 22.7 months and 17.4 months as well as the survival of 34 and 31 months in resected patients is very encouraging and better than historical controls.

2.5 Patients with borderline resectable pancreatic cancer are candidates for a prolonged course of systemic therapy followed by chemoradiation. Given the high rate of systemic relapse, the "most encouraging" systemic therapy currently available would be applicable in this situation. In the recent phase 3 study (NEJM), Conroy and colleagues reported on FOLFIRINOX superiority over gemcitabine in the treatment of advanced pancreatic cancer. The group randomly assigned 342 patients with an Eastern Cooperative Oncology Group performance status score of 0 or 1 (on a scale of 0 to 5, with higher scores indicating a greater severity of illness) to receive FOLFIRINOX or gemcitabine. Six months of chemotherapy were recommended in both groups in patients who had a response. The primary end point was overall survival. The median overall survival was 11.1 months in the FOLFIRINOX group as compared with 6.8 months in the gemcitabine group (hazard ratio for death, 0.57; 95% confidence interval [CI], 0.45 to 0.73; P<0.001). Median progression-free survival was 6.4 months in the FOLFIRINOX group and 3.3 months in the gemcitabine group (hazard ratio for disease progression, 0.47; 95% CI, 0.37 to 0.59; P<0.001). The objective response rate was 31.6% in the FOLFIRINOX group versus 9.4% in the gemcitabine group (P<0.001).

2.6 In this trial we propose systemic therapy with mFOLFIRINOX followed by chemoradiation for patients with upfront high risk resectable or borderline resectable pancreatic cancer to potentially maximize their chance of resectability and potentially improved disease free survival and overall survival after optimal preoperative therapy.
We plan to start with a 20% dose reduction and no 5-FU and leucovorin bolus for this trial (mFOLFIRINOX). Dropping the bolus decreases the toxicity and there is a perennial national shortage of leucovorin which makes it a challenge as well.

3.0 BACKGROUND DRUG INFORMATION

3.1 5FU

Fluorouracil injection, an antineoplastic antimetabolite, is a sterile, nonpyrogenic injectable solution for intravenous administration.

3.1.1 Chemistry

Chemically, fluorouracil, a fluorinated pyrimidine, is 5-fluoro-2,4 (1*H*,3*H*)-pyrimidinedione. It is a white to practically white crystalline powder which is sparingly soluble in water.

3.1.2 Clinical Pharmacology:

There is evidence that the metabolism of fluorouracil in the anabolic pathway blocks the methylation reaction of deoxyuridylic acid to thymidylic acid. In this manner, fluorouracil interferes with the synthesis of deoxyribonucleic acid (DNA) and to a lesser extent inhibits the formation of ribonucleic acid (RNA). Since DNA and RNA are essential for cell division and growth, the effect of fluorouracil may be to create a thymine deficiency which provokes unbalanced growth and death of the cell. The effects of DNA and RNA deprivation are most marked on those cells which grow more rapidly and which take up fluorouracil at a more rapid rate. Following intravenous injection, fluorouracil distributes into tumors, intestinal
mucosa, bone marrow, liver and other tissues throughout the body. In spite of its limited lipid solubility, fluorouracil diffuses readily across the blood-brain barrier and distributes into cerebrospinal fluid and brain tissue.
Seven percent to 20% of the parent drug is excreted unchanged in the urine in 6 hours; of this over 90% is excreted in the first hour. The remaining percentage of the administered dose is metabolized, primarily in the liver. The catabolic metabolism of fluorouracil results in degradation products (*e.g.*, CO2, urea and _-fluoro-_-alanine) which are inactive. The inactive metabolites are excreted in the urine over the next 3 to 4 hours.
Following intravenous administration of fluorouracil, the meanhalf-life of elimination from plasma is approximately 16 minutes, with a range of 8 to 20 minutes, and is dose dependent. No intact drug can be detected in the plasma 3 hours after an intravenous injection.

3.1.3 Human Toxicity:

Stomatitis and esophagopharyngitis (which may lead to sloughing and ulceration), diarrhea, anorexia, nausea and emesis are commonly seen during therapy.

Leukopenia usually follows every course of adequate therapy with fluorouracil. The lowest white blood cell counts are commonly observed between the 9th and 14th days after the first course of treatment, although uncommonly the maximal depression may be delayed for as long as 20 days. By the 30th day the count has usually returned to the normal range.
Alopecia and dermatitis may be seen in a substantial number of cases. The dermatitis most often seen is a pruritic maculopapular rash usually appearing on the extremities and less frequently on the trunk. It is generally reversible and usually responsive to symptomatic treatment.
Other adverse reactions are:
Hematologic: pancytopenia, thrombocytopenia, agranulocytosis, anemia.
Cardiovascular: myocardial ischemia, angina.
Gastrointestinal: gastrointestinal ulceration and bleeding.
Allergic Reactions: anaphylaxis and generalized allergic reactions.
Neurologic: acute cerebellar syndrome (which may persist following discontinuance of treatment), nystagmus, headache.
Dermatologic: dry skin; fissuring; photosensitivity, as manifested by erythema or increased pigmentation of the skin; vein pigmentation; palmar-plantar erythrodysesthesia syndrome, as manifested by tingling of the hands and feet followed by pain, erythema and swelling.
Ophthalmic: lacrimal duct stenosis, visual changes, lacrimation, photophobia.
Psychiatric: disorientation, confusion, euphoria.
Miscellaneous: thrombophlebitis, epistaxis, nail changes (including loss of nails).

3.1.4 Pharmaceutical Data: Each 10 mL contains 500 mg fluorouracil; pH is adjusted to approximately 9.2 with sodium hydroxide.
Chemically, fluorouracil, a fluorinated pyrimidine, is 5-fluoro-2,4 (1*H*,3*H*)-pyrimidinedione. It is a white to practically white crystalline powder which is sparingly soluble in water

3.1.5 Storage and Stability
Store at room temperature 15° to 30°C (59° to 86°F). Protect from light.

3.1.6 Route of Administration
Intravenous continuous infusion at fixed dose rate of 2400mg/m2 over 46 hours

3.1.7 Supplier
Commercially available

3.2 <u>Oxaliplatin</u>

3.2.1 Chemistry:
Oxaliplatin is an antineoplastic agent with the molecular formula C8H14N2O4Pt and the chemical name of cis-[(1R,2R)-1,2-cyclohexanediamine-N,N'] [oxalato(2-)-O,O'] platinum.

Oxaliplatin is an organoplatinum complex in which the platinum atom is complexed with 1,2-diaminocyclohexane (DACH) and with an oxalate ligand as a leaving group.

3.2.2 Clinical Pharmacology

Oxaliplatin undergoes nonenzymatic conversion in physiologic solutions to active derivatives via displacement of the labile

3.2.3 Human Toxicity

Neuropathy

Neuropathy was graded using a study-specific neurotoxicity scale, which was different than the National Cancer Institute Common Toxicity Criteria, Version 2.0 (NCI CTC) (see below). Oxaliplatin is associated with two types of neuropathy:

• An acute, reversible, primarily peripheral, sensory neuropathy that is of early onset, occurring within hours or one to two days of dosing, that resolves within 14 days, and that frequently recurs with further dosing. The symptoms may be precipitated or exacerbated by exposure to cold temperature or cold objects and they usually present as transient paresthesia, dysesthesia, and hypoesthesia in the hands, feet, perioral area, or throat. Jaw spasm, abnormal tongue sensation, dysarthria, eye pain, and a feeling of chest pressure have also been observed. An acute syndrome of pharyngolaryngeal dysesthesia seen in 1-2% of patients is characterized by subjective sensations of dysphagia or dyspnea, without any laryngospasm or bronchospasm (no stridor or wheezing).

• A persistent (>14 days), primarily peripheral, sensory neuropathy that is usually characterized by paresthesias, dysethesias, hypoesthesias, but may also include deficits in proprioception that caninterfere with daily activities (e.g. writing, buttoning, swallowing, and difficulty walking from impaired proprioception). Persistent neuropathy can occur without any prior acute neuropathy event.

• Pulmonary Toxicity

Oxaliplatin has been associated with pulmonary fibrosis (0.7% of study patients), which may be fatal. In case of unexplained respiratory symptoms such as non-productive cough, dyspnea, crackles, or radiological pulmonary infiltrates, Oxaliplatin should be discontinued until further pulmonary investigation excludes interstitial lung disease or pulmonary fibrosis.

3.2.4 Potential Drug-Drug Interactions

No pharmacokinetic interaction between 85 mg/m2 of Oxaliplatin and infusional 5-FU has been observed in patients treated every 2 weeks, but increases of 5-FU plasma concentrations by approximately 20% have been observed with doses of 130 mg/m2 of Oxaliplatin administered every 3 weeks. In vitro, platinum was not displaced from plasma proteins by the following medications: erythromycin, salicylate, sodium valproate, granisetron, and paclitaxel. In vitro, oxaliplatin is not metabolized by, nor does it inhibit, human cytochrome P450 isoenzymes. No P450-mediated drug-drug interactions are therefore anticipated in patients.

Since platinum-containing species are eliminated primarily through the kidney, clearance of these products may be decreased by co-administration of potentially nephrotoxic compounds, although this has not been specifically studied.

3.2.5 Pharmaceutical Data

Oxaliplatin is supplied in vials containing 50 mg or 100 mg of oxaliplatin as a sterile, preservative-free lyophilized powder for reconstitution. Lactose monohydrate is present as an inactive ingredient at 450 mg and 900 mg in the 50 mg and 100 mg dosage strengths, respectively.

3.2.6 Storage and Stability

RECONSTITUTION OR FINAL DILUTION MUST NEVER BE PERFORMED WITH A SODIUM CHLORIDE SOLUTION OR OTHER CHLORIDE-CONTAINING SOLUTIONS.

The lyophilized powder is reconstituted by adding 10 mL (for the 50 mg vial) or 20 mL (for the 100 mg vial) of Water for Injection, USP or 5% Dextrose Injection, USP. Do not administer the reconstituted solution without further dilution. The reconstituted solution must be further diluted in an infusion solution of 250-500 mL of 5% Dextrose Injection, USP. After reconstitution in the original vial, the solution may be stored up to 24 hours under refrigeration (2-8°C [36-46°F]). After final dilution with 250-500 mL of 5% Dextrose Injection, USP, the shelf life is 6 hours at room temperature (20-25°C [68-77°F]) or up to 24 hours under refrigeration (2-8°C [36-46°F]). Oxaliplatin is not light sensitive.

Oxaliplatin is incompatible in solution with alkaline medications or media (such as basic solutions of 5-FU) and must not be mixed with these or administered simultaneously through the same infusion line. The infusion line should be flushed with D5W prior to administration of any concomitant medication. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration and discarded if present.

3.2.7 Route of Administration

The recommended dose schedule given every two weeks is as follows:
Day 1: oxaliplatin 85 mg/m2 IV infusion in 250-500 mL D5W

3.2.8 Supplier

Commercially available

3.3. <u>Irinotecan</u>

3.3.1. Chemistry

Irinotecan Injection (irinotecan hydrochloride injection) is an antineoplastic agent of the topoisomerase I inhibitor class.

3.3.2 Clinical Pharmacology

Irinotecan is a derivative of camptothecin. Camptothecins interact specifically with the enzyme topoisomerase I which relieves torsional strain in DNA by inducing reversible

single-strand breaks. Irinotecan and its active metabolite SN-38 bind to the topoisomerase I-DNA complex and prevent religation of these single-strand breaks. Current research suggests that the cytotoxicity of irinotecan is due to double-strand DNA damage produced during DNA synthesis when replication enzymes
interact with the ternary complex formed by topoisomerase I, DNA, and either irinotecan or SN-38. Mammalian cells cannot efficiently repair these double-strand breaks.

Irinotecan serves as a water-soluble precursor of the lipophilic metabolite SN-38. SN-38 is formed from irinotecan by carboxylesterase-mediated cleavage of the carbamate bond between the camptothecin moiety and the dipiperidino side chain. SN-38 is approximately 1000times as potent as irinotecan as an inhibitor of topoisomeraseI purified from human and rodent tumor cell lines. In vitro cytotoxicity assays show that the potency of SN-38 relative to irinotecan varies from 2- to 2000-fold. However, the plasma area under the concentration versus time curve (AUC) values for SN-38 are 2% to 8% of irinotecan and SN-38 is 95% bound to plasma proteins compared to approximately 50% bound to plasma proteins for irinotecan (see Pharmacokinetics). The precise contribution of SN-38 to the activity of Irinotecan is thus unknown. Both irinotecan and SN-38 exist in an active lactone form and an inactive hydroxy acid anion form. A pH-dependent equilibrium exists between the two forms such that an acid pH promotes the formation of the lactone, while a more basic pH favors the hydroxyl acid anion form.

3.3.3 **Human Toxicity**

Overview of Adverse Events
Gastrointestinal: Nausea, vomiting, and diarrhea are common adverse events following treatment with CAMPTOSAR and can be severe. When observed, nausea and vomiting usually occur during or shortly after infusion of CAMPTOSAR. In the clinical studies testing the every 3-week-dosage schedule, the median time to the onset of late diarrhea was 5 days after irinotecan infusion. In the clinical studies evaluating the weekly dosage schedule, the median time to onset of late diarrhea was 11 days following administration of CAMPTOSAR. For patients starting treatment at the 125-mg/m2 weekly dose, the median duration of any grade of late diarrhea was 3 days. Among those patients treated at the 125-mg/m2 weekly dose who experienced grade 3 or 4 late diarrhea, the median duration of the entire episode of diarrhea was 7 days. The frequency of grade 3 or 4 late diarrhea was somewhat greater in patients starting treatment at 125 mg/m2 than in patients given a 100-mg/m2 weekly starting dose (34% [65/193] versus 23% [24/102]; p=0.08). The frequency of grade 3 and 4 late diarrhea by age was significantly greater in patients ≥65 years than in patients <65 years (40% [53/133] versus 23% [40/171]; p=0.002). In one study of the weekly dosage treatment, the frequency of grade 3 and 4 late diarrhea was significantly greater in male than in female patients (43% [25/58] versus 16% [5/32]; p=0.01), but there were no gender differences in the frequency of grade 3 and 4 late diarrhea in the other two studies of the weekly dosage treatment schedule. Colonic ulceration, sometimes with gastrointestinal bleeding, has been observed in association with administration of CAMPTOSAR.

Hematology: CAMPTOSAR commonly causes neutropenia, leukopenia (including lymphocytopenia), and anemia. Serious thrombocytopenia is uncommon. When evaluated in

the trials of weekly administration, the frequency of grade 3 and 4 neutropenia was significantly higher in patients who received previous pelvic/abdominal irradiation than in those who had not received such irradiation (48% [13/27] versus 24% [67/277]; p=0.04). In these same studies, patients with baseline serum total bilirubin levels of 1.0 mg/dL or more also had a significantly greater likelihood of experiencing first-cycle grade 3 or 4 neutropenia than those with bilirubin levels that were less than 1.0 mg/dL (50% [19/38] versus 18% [47/266]; p<0.001). There were no significant differences in the frequency of grade 3 and 4 neutropenia by age or gender. In the clinical studies evaluating the weekly dosage schedule, neutropenic fever (concurrent NCI grade 4 neutropenia and fever of grade 2 or greater) occurred in 3% of the patients; 6% of patients received G-CSF for the treatment of neutropenia. NCI grade 3 or 4 anemia was noted in 7% of the patients receiving weekly treatment; blood transfusions were given to 10% of the patients in these trials.

Body as a Whole: Asthenia, fever, and abdominal pain are generally the most common events of this type. Cholinergic Symptoms: Patients may have cholinergic symptoms of rhinitis, increased salivation, miosis, lacrimation, diaphoresis, flushing, and intestinal hyperperistalsis that can cause abdominal cramping and early diarrhea. If these symptoms occur, they manifest during or shortly after drug infusion. They are thought to be related to the anticholinesterase activity of the irinotecan parent compound and are expected to occur more frequently with higher irinotecan doses.
Hepatic: In the clinical studies evaluating the weekly dosage schedule, NCI grade 3 or 4 liver enzyme abnormalities were observed in fewer than 10% of patients. These events typically occur in patients with known hepatic metastases.

Dermatologic: Alopecia has been reported during treatment with CAMPTOSAR. Rashes have also been reported but did not result in discontinuation of treatment.
Respiratory: Severe pulmonary events are infrequent. In the clinical studies evaluating the weekly dosage schedule, NCI grade 3 or 4 dyspnea was reported in 4% of patients. Over half the patients with dyspnea had lung metastases; the extent to which malignant pulmonary involvement or other preexisting lung disease may have contributed to dyspnea in these patients is unknown.
Neurologic: Insomnia and dizziness can occur, but are not usually considered to be directly related to the administration of CAMPTOSAR. Dizziness may sometimes represent symptomatic evidence of orthostatic hypotension in patients with dehydration.

Cardiovascular: Vasodilation (flushing) may occur during administration of CAMPTOSAR. Bradycardia may also occur, but has not required intervention. These effects have been attributed to the cholinergic syndrome sometimes observed during or shortly after infusion of CAMPTOSAR. Thromboembolic events have been observed in patients receiving CAMPTOSAR; the specific cause of these events has not been determined.

3.3.4 Potential Drug-Drug Interactions

In a phase 1 clinical study involving irinotecan, 5-fluorouracil (5-FU), and leucovorin (LV) in 26 patients with solid tumors, the disposition of irinotecan was not substantially altered when the drugs were co-administered. Although the Cmax and AUC0-24 of SN-38, the active metabolite, were reduced (by 14% and 8%, respectively) when irinotecan was

followed by 5-FU and LV administration compared with when irinotecan was given alone, this sequence of administration was used in the combination trials and is recommended . Formal in vivo or in vitro drug interaction studies to evaluate the influence of irinotecan on the disposition of 5-FU and LV have not been conducted. Possible pharmacokinetic interactions of CAMPTOSAR with other concomitantly administered medications have not been formally investigated.

3.3.5 Pharmaceutical Data

Each mL of irinotecan Injection contains 20 mg irinotecan (on the basis of the trihydrate salt); 45 mg sorbitol; and 0.9 mg lactic acid. When necessary, pH has been adjusted to 3.5 (range, 3.0 to 3.8) with sodium hydroxide or hydrochloric acid.

Irinotecan injection must be diluted prior to infusion. Irinotecan should be diluted in 5% Dextrose Injection, USP, (preferred) or 0.9% Sodium Chloride Injection, USP, to a final concentration range of 0.12 to 2.8 mg/mL. The solution is physically and chemically stable for up to 24 hours at room temperature (approximately 25°C) and in ambient fluorescent lighting. Solutions diluted in 5% Dextrose Injection, USP, and stored at refrigerated temperatures (approximately 2° to 8°C), and protected from light are physically and chemically stable for 48 hours. Refrigeration of admixtures using 0.9% Sodium Chloride Injection, USP, is not recommended due to a low and sporadic incidence of visible particulates.

3.3.6 Storage and Stability

Store at controlled room temperature 15° to 30°C (59° to 86°F). Protect from light. It is recommended that the vial (and backing/plastic blister) should remain in the carton until the time of use.

3.3.7 Route of Administration

Intravenous administration at a dose of 180mg/m2 on day 1

3.3.8 Supplier

Commercially available

3.4 **Gemcitabine**

3.4.1 **Chemistry**

Gemcitabine HCl is 2-deoxy-2, 2-difluorocytidine monohydrochloride (beta isomer).

3.4.2 **Clinical Pharmacology**:

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. Clearance was affected by age and gender. Differences in either clearance volume or distribution based on patient characteristics or the duration of infusion results in changes in half-life and plasma concentrations.

The half-life of gemcitabine for short infusions ranged from 32-94 minutes, and the value for long infusions varied from 245-638 minutes, depending on age and gender, reflecting a greatly increased volume of distribution with longer infusions. The lower clearance in women and the elderly result in higher concentrations of gemcitabine for any given dose. The volume of distribution was increased with infusion duration. Volume of distribution of gemcitabine was 50 L/m^2 following infusions lasting <70 minutes, indicating that gemcitabine, after short infusions, is not extensively distributed into tissues. For longer infusions, the volume of distribution rose to 370 L/m^2, reflecting slow equilibration of gemcitabine within the tissue compartments. The maximum plasma concentrations of dFdU (inactive metabolite) were achieved up to 30 minutes after discontinuation of infusions, and the metabolite was excreted in urine without undergoing further biotransformation. The metabolite did not accumulate with weekly dosing, but its elimination is dependent on renal excretion, and could accumulate with decreased renal function. The effects of significant renal or hepatic insufficiency on the disposition of gemcitabine have not been systematically assessed.

3.4.3 **Human Toxicity**:

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. Diarrhea was reported by 19% of patients, and stomatitis by 11% of patients. *Hepatic*: gemcitabine was associated with 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 6/24 patients (0.25%) receiving gemcitabine in clinical trials. Four patients developed HUS on 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. 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:* Edema (13%), peripheral edema (20%), 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. 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. *Extravasation:* 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*: Two percent 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.

3.4.4 **Pharmaceutical Data**:
Gemcitabine is supplied as a lyophilized powder in sterile vials containing 200 mg or 1 g of gemcitabine as the hydrochloride salt (expressed as the free base), mannitol, and sodium acetate. Drug vials will be reconstituted with normal saline added to the vial to make a solution ideally containing 10 mg/mL or less. The concentration for 200 mg and 1 g vials should be nor greater than 40 mg/mL. An appropriate amount of drug will be prepared with normal saline and administered as a continuous infusion at 10mg/m2/min. Once the drug has been reconstituted, it should be stored at room temperature and used within 24 hours.

3.4.5 **Storage and Stability**
Store at controlled room temperature (20-25^{0}C), should be handled and disposed of in a manner consistent with other anti-cancer drugs.

3.4.6 **Route of Administration**
Intravenous infusion at fixed dose rate of 10mg/m2/min.

3.4.7 **Supplier**
Commercially available.

4.0 PATIENT ELIGIBILITY

4.1 INCLUSION CRITERIA:

4.1.1 Cytologic or histologic proof of adenocarcinoma of the pancreas is required prior to treatment. Patients with Islet cell tumors are not eligible.

4.1.2 Only untreated patients with high risk pancreatic adenocarcinomas will be eligible for the study. For this study, such patients are defined as those who meet one or more of the following radiographic or serologic criteria:

4.1.2.1 Primary tumor that involves the superior mesenteric vein causing a vein deformity or segmental venous occlusion with a patent vessel above and below suitable for reconstruction.

4.1.2.2 Primary tumor that involves $\leq$180 degrees of the superior mesenteric artery (SMA), celiac axis or any of its branches on CT or MRI

4.1.2.3 Primary tumor that abuts or encases ($\geq$ 50% of the vessel circumference) a short segment of the common hepatic artery (typically at the gastroduodenal artery origin)

4.1.2.4 Patients with a high CA19-9 (≥500mg/dl) in the presence of a bilirubin ≤2.0 mg/dL.

4.1.2.5 Radiographic findings consistent with malignant peripancreatic lymphadenopathy outside the planned field on CT or MRI

4.1.2.6 Radiographic findings of indeterminate liver or peritoneal lesions on CT orMRI concerning but not diagnostic of metastatic disease

4.1.3 Patients cannot have known hepatic or peritoneal metastases detected by ultrasound (US), CT scan, MRI or laparotomy.

4.1.4 There will be no upper age restriction; patients with Eastern Cooperative Oncology Group (ECOG) 0-1 are eligible (See Appendix B - ECOG Performance Status Scale).

4.1.5 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

4.1.6 Hepatic function (endoscopic or percutaneous drainage as needed)

- Total bilirubin ≤ 2 X institutional upper limits of normal (ULN)
- AST (SGOT)/ALT (SGPT) ≤ 5 X institutional ULN

4.1.7 Patients must have no fever or evidence of infection or other coexisting medical condition that would preclude protocol therapy.

4.1.8 Women of childbearing potential (defined as those who have not undergone a hysterectomy or who have not been postmenopausal for at least 24 consecutive months) must agree to practice adequate contraception and to refrain from breast feeding.

4.1.9 Patients must sign a study-specific consent form, which is attached to this protocol.

4.2 EXCLUSION CRITERIA:

4.2.1 Patients whose tumors are defined as locally advanced cancer or metastatic cancer are not eligible.

4.2.2 Unstable angina or New York Heart Association (NYHA) Grade II or greater congestive heart failure; multiple comorbidity that preclude a major abdominal surgery

4.2.3 Known presence of metastases

4.2.4 Inability to comply with study and/or follow-up procedures

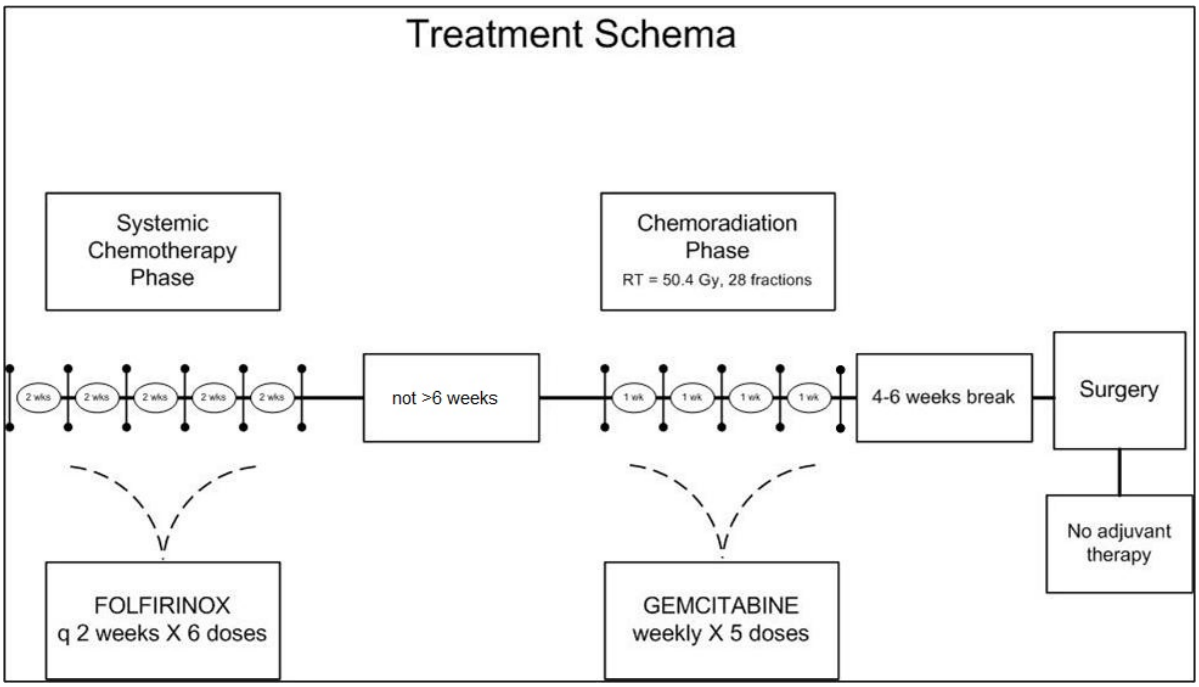
4.2.5 Patients < 18 years of age.

4.2.6 Pregnant women with a positive (blood B-HCG) pregnancy test are excluded from this study;

4.2.7 Patients with an active second malignancy with the exception of non-melanoma skin cancer.

5.0 TREATMENT PLAN

5.1 Study Schema: We have acquired extensive experience with the mFOLFIRINOX regimen and believe the doses as published in ACCORD11 can be toxic with significant hematologic toxicities, especially in patients with pancreatic head tumors and stent in place. We plan to start with a 20% dose reduction from the reported doses of FOLFIRINOX and no 5-FU and leucovorin bolus for this trial (mFOLFIRINOX). Dropping the bolus decreases the toxicity and there is a perennial national shortage of leucovorin which makes it a challenge as well. Treatment schema for the various phases of the study shown below:



SYSTEMIC PHASE: Chemotherapy with Oxaliplatin followed by Irinotecan followed by 5Fluorouracil.

m FOLFIRINOX - oxaliplatin 75 mg/m^2 d1 + irinotecan 150 mg/m^{2*} d1 + 5-fluorouracil 2,000 mg/m^2 46h continuous infusion, every other week for 6 cycles (12 weeks). After calculation, doses can be 'rounded" to a whole number for safety.

Agent	Dose	Route	Schedule
Oxaliplatin	75 mg/m^2 in D5W 500ml	IV infused over 2 hours.	Day 1
Irinotecan*	150 mg/m^2 in D5W 500ml	IV infused over 90 minutes	Day 1
5fluorouracil	2000 mg/m^2	IV 46 hour continuous infusion via an ambulatory pump	Day 1-2

*Patients with suspected Gilbert's syndrome may receive 25-50% of this dose of irinotecan based on physician discretion for all cycles.

CHEMORADIATION PHASE: This phase will start no more than 6 weeks after completion of the last cycle of mFOLFIRINOX. The 6 weeks may be extended at the discretion of the principal investigator. Chemoradiation with

Gemcitabine: 350 mg/m^2 IV over 35 minutes every week for 5 doses beginning day 1 (days 1, 8, 15, 22, 29)

Radiation: External beam radiation therapy will be delivered 5 days/week +/- 2 days over 5.5 weeks with 18-MeV photons. 3D conformal RT, a total dose of 50.4 Gy prescribed to the 95% isodose at 1.8 Gy/fraction (28 fractions) to the GTV + 1.5 cm margin. If any days of radiation are missed, these days may be made up at the end of treatment so that the patient will receive the full amount of radiation.

(CA 19-9 and Restaging scans will be performed at the end of the systemic phase and chemoradiation phases of the study)

SURGERY– At least 4-6 weeks after the last dose of Gemcitabine if there is no local progression or distant metastasis. Patients whose scans show unequivocal local or distant progression are not candidates for surgery

Postoperative Therapy: All patients will get postoperative restaging scans after surgery as per institutional standard of care guidelines. No postoperative adjuvant therapy is planned in this study.

Schedule of Assessments

	Baseline - within 6 wks prior to start of tx.	Systemic Phase						Recovery ≤ 6 weeks from end of Systemic Therapy	Chemo-radiation Phase						Surgery [4-6 wk post Chemo-XRT]	2-3 months post surgery	Postop
		wk1	wk3	wk5	wk7	wk9	wk11		wk1	wk2	wk3	wk4	wk5	wk6			
Physical Exam	X	X[f]	X[f]	X[f]	X[f]	X[f]	X[f]		X[f]	X[f]	X[f]	X[f]	X[f]	X[f]			
Imaging/Restaging	X								X[a]						X[b]		x[h]
Cytology/histology	X																
CA19-9	X	X[j,f]							X[a]						X[b]		
ECOG Performance Status	X																
Vital Signs[c]		X	X	X	X	X	X		X	X	X	X	X	X			
CBC[d]	X	X[f]	X[f]	X[f]	X[f]	X[f]	X[f]		X[f]	X[f]	X[f]	X[f]	X[f]	X[f]			
Chemistries[e]	X	X[f]	X[f]	X[f]	X[f]	X[f]	X[f]		X[f]	X[f]	X[f]	X[f]	X[f]	X[f]			
Serum pregnancy test for WOCBP	X																
mFOLFIRINOX [K]		X	X	X	X	X	X										
XRT [i]									X	X	X	X	X	X			
Gemcitabine[n]									X	X	X	X	X				
Surgery															X		
CTC[g]	X													X[l]	X[m]	X	
Correlative–tissue sample															X		

a. within 4 weeks prior to start of treatment

b. at least 4 weeks post chemoxrt treatment but prior to surgery

c. to include height [baseline only], weight, blood pressure, pulse

d. CBC and platelet count

e. Chemistries include BUN, Creatinine, total bilirubin, AST, ALT, alkaline Phosphatase, phosphorus, calcium, glucose, total

protein, albumin, sodium, potassium, CO^2 , chloride and magnesium	
f. up to 7 days prior to treatment for week 1 of systemic and chemoradiation phase; for the remaining weeks up to 3 days prior to treatment	
g. from the first 30 eligible and consenting patients that begin treatment	
h. After completion of surgery, CT scan or MRI of the abdomen and pelvis will be done every four months (+/- 28 days) for two years.	
i. External beam radiation therapy will be delivered 5 days/week (Monday-Friday) over 5.5 weeks. If any days of radiation are missed, these days may be made up at the end of treatment so that the patient will receive the full amount of radiation.	
j. Optional – may be performed at the discretion of the investigator. k. Window, +/- 2 days for treatment except week 1 l. Post treatment – pre-surgery CTC collection (within 2 weeks of surgery including day of surgery) m. Intraoperative CTC collection is optional for the surgeon based on safety and feasibility of portal vein blood draw. n. Window, +/- 1 day for treatment except week 1	

5.2 Dose Adjustments:

5.2.1 Dose adjustment for Preoperative Chemotherapy: Dose Adjustments of mFOLFIRINOX on Subsequent Treatment Cycles after the toxicity has decreased to grade 1 or less (Based on the worst toxicity due to chemotherapy on any day of the previous cycle, NCI- Common Toxicity Criteria v.4).

Table 1: Hematologic Toxicities - Dose Adjustment

ANC ($X10^3$/L)- as noted in previous cycle	Platelet ($X10^9$/L)- as noted in previous cycle		Percent dose of mFOLFIRINOX[1] for subsequent cycle based on previous
≥ 0.91	And	≥75	100
0.51-0.90	Or	51-74	75
≤0.5	Or	≤50	50

1. Percent dosage refers to the dose of drugs on the first day of the cycle.
2. Patients with ANC </= 0.5 will receive pegfilgrastrim (6 mg SQ on day 3 or 4 of chemotherapy). In all other patients, use of pegfilgrastrim is up to the discretion of the physician (for initial and subsequent cycles of therapy).

5.2.2 Dose Adjustments for Non-Hematological Toxicity

Table 2: Non-Hematologic Toxicities - Dose Adjustment

Worst toxicity during cycle	Percent dose of mFOLFIRINOX[1]
0/1	100
2	75
3-4	50

1. Percent dosage refers to the dose of drugs on the first day of the cycle

Note:

1. The physician should keep reducing the doses (25% reduction) in subsequent cycles if still faced with significant hematological or non hematological toxicity
2. Treatment between cycles may be held for up to 4 additional weeks (total 6 weeks between cycles) if the patient has not recovered to grade 1 or less hematologic or non hematologic toxicity prior to subsequent cycle.
3. If deemed to be in the best interest of the patient, at the investigator's discretion, one of the 3 drugs may be reduced as per table (instead of all 3 drugs) for ≥ grade 2 non-hematologic toxicity.

5.2.3 Dose adjustment for specific toxicities

Table 3. Dose adjustment for 5FU and irinotecan for Diarrhea (despite optimal use of loperamide). No change in oxaliplatin dose

Worst toxicity	Dose of 5FU and Irinotecan
Grade 1	Maintain previous dose
Grade 2	75% of previous dose
Grade 3 or 4	50% of previous dose

Table 4. Dose adjustment for oxaliplatin for neurotoxicity. No change in 5FU and irinotecan doses.

Worst toxicity	Dose of oxaliplatin
Grade 1	Maintain previous dose
Grade $\geq$ 2	Reduce by one dose level (i.e. 25% dose reduction from last dose/ cycle)

5.2.4 Dose adjustment for Preoperative Gemcitabine and Concurrent RT (chemoradiation phase)

Table 5 shows the dose adjustments for gemcitabine during chemoradiation and counts will be monitored every week during chemoradiation. Nonhematologic toxicities will be assessed and doses adjusted accordingly (Table 6).

Table 5: Hematologic Toxicities - Dose Adjustment of Gemcitabine During Gemcitabine/XRT*

ANC ($X10^3$/L)	Platelets (/mL)	Percent of Full Dose
$\geq$1.0 and	>100,000	100
0.51-0.99 or	50,000-100,000	75
$\leq$ 0.5 or	<50,000	Hold

*Previous experience suggests myelosuppression will not require holding radiation.

Table 6: Nonhematologic Toxicities - Dose Adjustment of Gemcitabine During Gemcitabine/XRT

Grade	Percent of Full Dose
0-1	100
2	75(
3-4	Hold
Hospitalization	Hold

Note: For grade 3-4 toxicity, gemcitabine to be restarted after toxicity drops to ≤ grade 2 at 75% of the previous dose

Gemcitabine Dose Modifications
Missed doses of gemcitabine will be omitted rather than made up. If multiple toxicities are seen, administer dose based on greatest reduction required for any single toxicity observed. Once reduced, no dose re-escalation of gemcitabine is allowed

Two dose reductions are allowed. If a third reduction is required, patient should discontinue gemcitabine unless the investigator deems that continued treatment is in the best interest of the patient.

5.3 Standard hydration/antiemetic regimen

IV hydration required at the medical or radiation oncologist's discretion. Standard of care orders for premedications and hydration at the discretion of the medical oncologist.

5.4 Radiation Therapy

5.4.1 Target Volume and Dose:
External beam radiation therapy will be delivered 5 days/week +/- 2 days over 5.5 weeks with 18-MeV photons. 3D conformal RT, a total dose of 50.4 Gy prescribed to the 95% isodose at 1.8 Gy/fraction (28 fractions) to the GTV + 1.5 cm margin. If any days of radiation are missed, these days may be made up at the end of treatment so that the patient will receive the full amount of radiation.

5.4.2 All patients will receive RT per informed consent and will receive AP/PA and lateral fields to the pancreas with customized blocking. Simulation should be done with the patient in the supine position with “arms up” position. If any days of radiation are missed, these days may be made up at the end of treatment so that the patient will receive the full amount of radiation.

5.4.3 A CT scan, excretory urogram, or renal nuclear medicine scan must be done before the start treatment to define renal function, as at least 2/3 of one kidney needs to be excluded from all irradiation fields.

5.5 Surgery

5.5. 1 Surgery will be planned at least 4 weeks after radiation therapy.

In the absence of metastases, tumor mobilization and surgical resection will be performed as necessary based on standard of care surgery guidelines. Feeding jejunostomy and gastrostomy tubes may be placed at the discretion of the operating surgeon and are part of standard of care management.

~~5.6.3~~ 5.6 Pathology

Margins assessed: pancreatic, biliary, uncinate and duodenal based on surgical plan and standard of care. All standard of care pathologic data will be recorded.

6.0 PRETREATMENT EVALUATION

Within 6 weeks Prior to Study Enrollment: Patients must have appropriate lab and radiographic studies (CXR; CT abdomen and pelvis or MRI; CBC; platelet count; BUN; creatinine; bilirubin; AST, ALT, Ca19-9) conducted prior to study enrollment to meet eligibility criteria. Patient also must have undergone a (baseline) history and physical examination, evaluation of concomittant medications, weight, height, performance status measurement.

7.0 EVALUATION DURING PREOPERATIVE TREATMENT

During the chemotherapy phase, the attending physician must see each patient every two weeks within 3 days prior to each dose of chemotherapy.

The patient will be assessed as follows:

7.1 Complete blood count (CBC: hemoglobin, hematocrit, red blood cells [RBC], WBC, platelets, and differential blood cell counts (neutrophils, bands, lymphocytes, monocytes, eosinophils, basophils) within 3 days prior to each dose of mFOLFIRINOX.

7.2 Blood chemistries (bilirubin, alkaline phosphatase, ALT, AST, BUN, creatinine, phosphorus, calcium, glucose, total protein, albumin, and electrolytes [sodium, potassium, CO^2, chloride, and magnesium]) Within 3 days prior to each dose FOLFIRNOX.

7.3 Vital signs (blood pressure and pulse rate) on days of infusion treatment.

During chemoradiation phase, the attending physician must see each patient every week (1-3 days prior to each dose of gemcitabine) with weekly CBC and chemistries .

8.0 EVALUATION AFTER COMPLETION OF SURGERY

8.1. The primary endpoint in this study is resection rate. Survival will be followed as a secondary endpoint, as measured from the time of histologic or cytologic diagnosis of pancreatic adenocarcinoma. Patterns of tumor recurrence will be assessed by obtaining surveillance imaging based on standard of care guidelines.

8.2 After completion of surgery, CT scan or MRI of the abdomen and pelvis will be done every four months (+/- 28 days) for two years.

9.0 CRITERIA FOR DISCONTINUING THERAPY

- Inability of patient to comply with study requirements
- Determination by the investigator that it is no longer safe for the patient to continue therapy
- Unresectable or metastatic disease after chemoradiation.
- Subjects withdraws consent.

10. Correlative studies

10.1 Tissue based: DPC4 (SMAD4) status determination – preoperative (on aspiration and biopsy) and after surgery (pathologic tissue)

Background data: Recent rapid autopsy data presented by Dr. Iacobuzio-Donahue and colleagues suggest that pancreatic cancers can present with distinct genetic subtypes with different patterns of failure. In this study, patients with DPC4 intact tumors were more likely to die of locally destructive disease (30% of patients) and those with DPC4 mutated tumors with a distant widespread metastatic disease (70%). These distinct patterns of failure (locally destructive versus metastatic) were unrelated to clinical stage at presentation, treatment history, and histopathologic features. There is significant interest in understanding if this data holds true in patients being treated (prospectively) and eventually use this information to guide therapy based on sub-groups of patients (locally destructive or wildly metastatic phenotypes). The feasibility of determining DPC4 status on diagnostic cytology specimens was tested recently at our institution in patients with locally advanced pancreatic cancer using immunohistochemical staining.

We will collect preexisting fine needle aspiration and biopsy specimens (up to 4 unstained slides) when available from patients diagnosed with pancreatic cancer at MDACC

(preoperative) and their final pathology tissue (from pancreas tissue bank, up to 4 unstained slides). Immunohistochemistry for DPC4 will be performed on this tissue by Dr. Huamin Wang from pathology. This will not impact the therapy decision which is independent of the correlative studies.

10.2 Circulating tumor cells measurements: Preliminary study to evaluate the feasibility of measuring CTCs and the role of these cells as a prognostic and/or predictive marker in borderline resectable adenocarcinoma of the pancreas.

There is an unmet research need to access pancreatic cancer CTCs from the blood given that biopsy of pancreatic cancerous tissue is a significant risk and requires invasive procedures. There is an urgent need to improve the diagnosis and treatment of pancreatic cancer patients by providing relatively painless, minimally invasive access to patients' cancer cells from a blood sample. In addition, we are keen to evaluate a technology that can detect very low concentrations of target cellular material, with the goal of enabling clinicians to discover hard-to-detect cancers at a very early stage. CTCs are rare, usually less than 10 cells in one milliliter (ml) of blood, making their detection and analysis very difficult by current methods. The Veridex CellSearch® CTC IVD test which uses an immunomagnetic method has not been able retrieve optimal pancreatic CTCs from the blood and not proven to be a prognostic marker in pancreatic cancer.

For isolating CTCs in the blood, we will collaborate with ApoCell, and evaluate its new technology (Apostream ™) that may improve the detection of pancreatic tumor cells circulating in the blood. Invented by scientists at The University of Texas MD Anderson Cancer Center's Laboratory of Diagnostic Microsystems this technology may enable the capture of pancreatic CTCs in a live and viable state, enabling post detection testing and culturing.

The Apostream ™ technology is based on cell morphology and exploits the fundamental differences in form and structure between healthy cells and cancer cells. By exposing the patient's blood sample to a low level electrical field of varying frequencies, enabling us to separate the cancer cells, based on their inherently different properties, from all other cells. This process is called dielectrophoresis field flow fractionation (DEP-FFF). We are hopeful that with this technology, if we are able to capture sufficient viable pancreatic CTCs, it will allow for protein biomarker analysis, mutation detection and culturing of these rare cells for *in vitro* drug testing to accelerate the drug development process.

Blood will be drawn at 4 time points from 30 patients:

- pretreatment (baseline),
- post treatment-presurgery (within 2 weeks of surgery including day of surgery)
- during surgery (portal vein blood) and
- 2-3 months after surgery.

Patients will be consented for all the time points as shown above, although intra-operative CTC collection (time point # 3) is optional for the surgeon based on safety and feasibility of portal vein blood draw during pancreatectomy.

This pilot study will include a total of 30 patients to quantify CTCs by theApoStream™ system. In the first 10 patients, in addition to the ApoStream™ methodology,quantification of CTCs will also be performed by the CellSearch® platform, and the two techniques will be compared.

The first ten (10) consented individuals will each have two (2) blood tubes drawn: 8 mL BD Vacutainer® CPT Citrate Blood Collection tube, and 10 mL CellSave® blood tube.

The subsequent twenty (20) consented individuals will each have one (1) 8 mL BD Vacutainer® CPT Citrate Blood Collection tube drawn. ApoCell, Inc.will serve as the laboratory for analytical procedures.

Analyses:
First 10 patients: Blood will be subjected to CTC enrichment using CellSearch® CTC kit (CellSave® tube) and the ApoStream™ platform (CPT tube). CTCs isolated by CellSearch® will be analyzed using the CellTracks® Analyzer II, and the final CTC images will be scored by a Veridex, LLC certified analyst. CTC enumeration will be reported as number of EpCAM enriched, cytokeratin CK+, CD45- and DAPI+ cells per 7.5mL of blood.
CTCs isolated by the CTCs isolated by ApoStream™ platform will be enumerated following immunofluorescent staining with antibodies against Cytokeratin (CK), CD45, and CA-19-9 (pancreatic cell marker). The following will be analyzed and reported: 1) total number of CA-19-9+/CD45-/DAPI+ cells, 2) total number of CK+/CD45-/DAPI+ cells, 3) total number of CK-/CD45-/DAPI+ cells , and 4) total number of CK+/CD45+/DAPI+ cells.

Remaining 20 patients: CTCs isolated on the ApoStream™ platform will be enumerated following immunofluorescent staining with antibodies against Cytokeratin (CK), CD45, and CA-19-9 (pancreatic cell marker). The following will be analyzed and reported: 1) total number of CA-19-9+/CD45-/DAPI+ cells, 2) total number of CK+/CD45-/DAPI+ cells, 3) total number of CA-19-9+/CK+/CD45-/DAPI+ cells. We will also plan to evaluate for Kras mutation and SMAD4 deletions in the CTCs of these 20 patients and compare it to the pancreas cancerous tissue (preoperative specimen when available and the pathologic specimen).

Any residual blood will be discarded. Slides with cells extracted from patient blood will be discarded.

Note: If during the processing phase, any of the tubes break, we will continue to proceed with drawing the other time points and proceed with analyses of the intact tubes (ones already drawn). Also, if the patient does not proceed with surgery (and we are able to get tubes for the 2 time points prior to surgery, these will be analyzed). Also, if at any time point (including first), if blood not drawn for CTC, CTC can still be drawn at remaining time points

10.3 Imaging correlative study

We have previously developed quantitative imaging biomarkers from standard of care computed tomography (CT) scans (Koay et al, JCI 2014). We will apply these methods to the CT scans in this study as a correlate of response and survival outcomes. Standard statistical tests will be used to describe the potential correlations, including chi-squared test for associations of the imaging biomarkers with pathological measurements, and the log rank test for associations with survival outcomes such as progression free survival and overall survival.

11.0 STATISTICAL CONSIDERATIONS

This is a single-center single-arm, phase II study to assess resectability rate in patients treated with preoperative mFOLFIRINOX chemotherapy followed by Gemcitabine based chemoradiation therapy for patients with borderline resectable adenocarcinoma of the pancreas. Patients will receive mFOLFIRINOX chemotherapy for 6 cycles (12 weeks) followed by chemoradiation therapy (5 doses of gemcitabine and 6 weeks of radiation therapy). Patients will be restaged at the end of systemic chemotherapy as well as chemoradiation therapy and may drop off the study due to disease progression or metastasis. At least 4-6 weeks after the last dose of Gemcitabine, patients may undergo surgery if no local disease progression or distant metastasis is detected during a final restaging scan. Patient resectability rate is defined as the proportion of patients who undergo surgery among all enrolled patients. A total of 33patients will be enrolled into the study, at a rate of 1-2 patients per month.

Statistical Design

We will apply the Simon's optimum two-stage design in this study. A sample size of 33 is chosen to differentiate between a good resectability rate of 60% and a poor resectability rate of 40% with 80% power and at a significance level of 0.10. The trial will stop early due to lack of efficacy if there are 7 or fewer patients undergo surgery among the first 16 patients. Otherwise, the study will continue to enroll the remaining 17 patients. By the end of the trial, lack of efficacy will be claimed if 16 or fewer patients undergo surgery among the total 33 enrolled patients. Under the null hypothesis of a poor resectability rate of 40%, the probability of early stopping is 71.6% and the expected sample size is 21 patients.

Toxicity will be continuously monitored throughout the study, which includes grade 4 hematological toxicity and Grade 3 or higher non-hematological toxicity. A beta (0.4, 0.6) prior was assumed for the distribution of the toxicity rate. Starting from the 6^{th} patient, we will apply the following toxicity monitoring rule every 3 patients.

$$\Pr(\text{Tox} > 40\% \mid \text{data}) > 90\%$$

The rule implies that we will stop the trial if any time, there is more than 95% probability that the toxicity rate is more than 40%. The corresponding stopping boundaries are to stop the trial if [# patients with toxicity] / [# evaluable patients] >/= 5/6, 6/9, 8/12, 9/15, 10/18, 12/21, 13/24, 15/27, 16/30. The operating characteristics corresponding to this toxicity monitoring rule is shown in Table 7.

Table 7. Operating characteristics for toxicity monitoring.

True toxicity rate	Prob(stop the trial early)

0.2	0.005
0.3	0.048
0.4	0.231
0.5	0.588
0.6	0.892

Statistical analysis
The resectability rate will be estimated, along with the 95% confidence interval, using the intent-to-treat (ITT) principle. For patients who undergo surgery, the proportion of margin positive resection (R1) and margin negative resection (R0) will be estimated, along with the 95% confidence interval. Kaplan-Meier method will be used to assess overall survival (OS), progression-free survival (PFS) and disease-free survival (DFS). The pattern of local and distant failure will be summarized through frequency and percentage. In addition, we will assess the status of DPC4 (positive versus negative) preoperative and after surgery and to evaluate the association between the DPC4 status and survival endpoints. CTCs will also be measured from blood drawn at baseline, post treatment-presurgery, during surgery and 2-3 months after surgery. Analysis of CTCs will be reported by data tabulations, descriptive statistics, and graphical presentations and as appropriate in relation to survival endpoints.

12.0 DATA AND PROTOCOL MANAGEMENT

12.1 After obtaining informed consent**,** patients will be registered on the MDACC computerized Clinical Oncology Research System (CORe).

12.2 The attending physician must see each patient before each dose of chemotherapy.

12.3 Data Collection for Enrolled patients
Designated research personnel must enter the information required by the protocol onto electronic Case Report Forms (CRFs). The University of Texas M D Anderson Cancer Center's Clinical oncology Research (CORE) system, and the University of Texas MD Anderson Cancer Center's Protocol Data Management System©(PDMS) CRF system will be used for this study. PDMS is a clinical research information management system. The PDMS CRF is an electronic document designed to record all the protocol-required information to be reported on each trial subject.
PDMS provides data entry templates as defined in the protocol. Laboratory results are automatically transferred from M. D. Anderson Cancer Center Laboratory Medicine's server to PDMS each morning. Users must have clearance through the M. D. Anderson Cancer Center Information Services Security Department in order to access PDMS. PDMS login is password protected.

Only adverse events that are grade 1, 2,3,4,5 possible, probable or definite will be recorded.The Investigator is responsible for verifying and providing source documentation for all adverse events and assigning attribution for each event for all subjects enrolled on the trial.

13.0 ADVERSE EVENT REPORTING REQUIREMENTS

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

13.2 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).

APPENDIX A

Table 8. Comparison of the past neoadjuvant clinical trials at University of Texas, M. D. Anderson Cancer Center

	5-FU 50.4 Gy	5-FU 30 Gy	Paclitaxel 30 Gy	Gem-XRT	Gem-Cis XRT
No. of Patients	28	35	37	86	90
Overall survival (mo)	NA	NA	12	23	17
No. Hospitalized prior to restaging (%)	9 (32)	1 (1)	6/35 (17)	36(42)	*40 (51)
No. who completed all treatment including PD (%)	17(60)	20(57)	20(54)	64 (74)	*52 (66)
No. Histologic response IIB-IV/total resected (%)	7 (41)	4 (20)	4/19 (21)	37(58)	31 (60)
No. SMA margin positive (%)	3 (18)	2 (10)	6/19 (32)	4(6)	1 (2)
No. Death during treatment (%)	1 (4)	0	0	1 (1)	**1 (1)
Median survival of patients who completed all treatment (mo)	NA	25	19	34	31
Median survival of patients who did not complete all treatment (mo)	NA	7	10	7.1	10.5
No. local recurrence (%)	NA	1 (5)	0	7 (11)	13 (25)
No. Isolated local recurrence (%)	NA	1 (5)	0	2 (3)	3 (6)

* Out of the 79 patients who completed preoperative therapy
** This patient was one of the 11 patients who withdrew from treatment prior to re-staging

Table 9 - Comparison of the last two neoadjuvant clinical trials

	Gem-XRT	Gem-Cis-XRT	P-value
Total No. Patients	86	*79	-
Median Age	65	65	0.85
No. Smokers (%)	59 (69)	48 (61)	0.29
No. Previous Laparotomy (%)	8 (9)	4 (5)	
No. with biliary stent (%)	67 (78)	64 (81)	0.62
No. with Metal stent (%)	2 (2)	36 (46)	<.001
No. Hospitalized (with grade 3-4 toxicity) prior to restaging v	38 (44)	40 (51)	0.26
No. Grade 3 or 4 toxicity (%)	74 (86)	63/79 (80)	0.28
No. who completed all treatment to include PD (%)	64 (74)	52 (66)	0.23
No. N1 (%)	24 /64 (38)	31/52 (60)	0.01
No. T3 (%)	43/64 (67)	43/52 (83)	0.04
No. Vascular resection (%)	13/64 (20)	19/52 (36)	0.05
No. SMA margin positive (%)	4/64 (6)	1/52 (2)	0.38
No. Histologic response IIB-IV (%)	37/64 (58)	31/52 (60)	0.75
No. Death during treatment (%)	1	0	
Median survival of all patients (months)	22.7	17.4	0.08
Median survival of patients who	34.0	31.0	0.41

completed all treatment (months)			
Median survival of patients who did not complete all treatment (months)	7.1	10.5	0.12
No. isolated local failure (%)	2/64 (3)	3/52 (6)	0.66

* 79 patients in the current report completed preoperative therapy.

Appendix B:

ECOG PERFORMANCE STATUS*	
Grade	**ECOG**
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair
5	Dead

* As published in Am. J. Clin. Oncol.: *Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.*

Appendix C:

University of Texas M. D. Anderson Cancer Center
Institutional Review Board Policy on
Reporting Serious Adverse Events

DEFINITIONS	**Adverse Event (AE**) – Any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the subject's participation in the research. **Expected AE** - Any AE with specificity or severity that is consistent with the current Investigator Brochure (IB) or consistent with the risk information described in the Informed Consent Document (ICD) or general investigational plan. All clinical protocols should include a list of the expected and anticipated events or hospitalizations relating to the study treatment. **Unexpected (Unanticipated) AE** - Any AE, with specificity or severity that is not consistent with the current IB or not consistent with the risk information described in the ICD or general investigational plan. **Serious Adverse Event (SAE)** – Any AE associated with the subject's participation in research that: • results in death; • is life-threatening, (places the subject at immediate risk of death from the event as it occurred); • results in inpatient hospitalization or prolongation of existing hospitalization; • results in persistent or significant disability/incapacity; • results in a congenital anomaly/birth defect; or • based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the other outcomes listed in this definition. **Internal AE** - An AE occurring in a subject who is enrolled in a protocol or study that is under the oversight of a UTMDACC IRB. **Related:** Events directly or indirectly attributed to study drug, device or procedures and/or study participation. Events occurring with sufficient frequency to suggest that they are not random.

	Unrelated: Events that would occur regardless of study participation, including events that are clearly random occurrences. If the frequency of the event suggests a possible connection to the study intervention, then it should be considered related. **AE Attribution** - The determination of whether an AE is related to the research (medical treatment or intervention): Definite – It is clearly related Probable - It is likely related Possible - It may be related Unlikely - It is doubtfully related Unrelated - It is clearly NOT related **AE Severity** - Refers to the intensity (grading) of a specific AE. For the purpose of this policy, toxicity is synonymous with AE.

Institutional Procedures

Reporting Requirements for Internal AEs	**Serious Internal AEs (SIAEs) requiring prompt reporting to the IRB** All internal AEs that include all of the following will require prompt reporting to IRB via the Office of Protocol Research (OPR): • Serious • Unexpected • Related (definitely, probably, or possibly related) to participation in the research. Timeline for prompt reporting: 1. **Within 1 working day (24 hours) from the time the research team becomes aware of the event** = Deaths that are unexpected and definitely, probably or possibly related to study intervention that occur during and within 30 days after the last day of active study intervention. 2. **Within 5 working days from the time the research team becomes aware of the event** = All other serious, unexpected and definitely, probably or possibly related AEs. SAEs will require prompt reporting from the time protocol specific consent is signed, and screening has begun, during the course of study intervention and within 30 days after the last day of active study intervention. Beyond 30 days of study intervention, completion of only those SAEs that,

	in the judgment of the investigator, are related to research will require prompt reporting. **<u>SIAEs Requiring Documentation on AE Log</u>**: All SIAEs that do not fall under the prompt reporting requirements should be reported during continuing review using the Internal SAE Log or database printout. **<u>Note:</u>** If a trend or recurring adverse events are seen, the PI should report these events using the Internal SAE report Form for prompt reporting. **Individual reports submitted to IRB that do not meet the requirements for prompt reporting will be returned to PI.** NOTE: Certain types of incidents, experiences, and outcomes that occur during the conduct of research are unexpected, related or possibly related to the research, and place the research subject at a greater risk of harm but are not considered adverse events. These incidents are classified as unanticipated problems and will require reporting to the IRB. These have different reporting forms and are discussed under the "Policy on Reporting Protocol Deviations, Protocol Violations, and Unanticipated Problems." This policy does not replace the reporting requirements to the FDA, the sponsor or monitoring agency.

Appendix D

New York Heart Association Classification of Cardiac Disease

Class	Functional Capacity	Objective Assessment
I	Patients with cardiac disease but without resulting limitations of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea, or anginal pain.	No objective evidence of cardiovascular disease.
II	Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea, or anginal pain.	Objective evidence of minimal cardiovascular disease.
III	Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes fatigue, palpitation, dyspnea, or anginal pain.	Objective evidence of moderately severe cardiovascular disease.
IV	Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of heart failure or the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort is increased.	Objective evidence of severe cardiovascular disease.

Appendix E—ApoCell Lab Analysis

Blood will be drawn at 4 time points from 30 patients:

- pretreatment (baseline),
- post treatment-presurgery (within 2 weeks of surgery including day of surgery)
- during surgery (portal vein blood) and
- 2-3 months after surgery.

The first ten (10) consented individuals will each have two (2) blood tubes drawn: 8 mL BD Vacutainer® CPT Citrate Blood Collection tube, and 10 mL CellSave® blood tube.

The subsequent twenty (20) consented individuals will each have one (1) 8 mL BD Vacutainer® CPT Citrate Blood Collection tube drawn. Coded blood collection tubes, Blood Collection Instructions and Specimen Submission Instruction forms will be provided by ApoCell, Inc., who will serve as the laboratory for analytical procedures.

ApoCell, Inc.
2575 West Bellfort Street. Suite 190
Houston, Texas 77054.

Blood Collection Instructions

- Check the expiration date on the CPT and CellSave® tube before use.
- Do NOT collect blood on weekends.
- Notify both Courtney Williams and Apocell Logistics team by email 24 hours prior to blood draw.
- Contact Courtney Williams by phone as primary and if not available, Apocell Logistics as back up prior to and immediately after blood sample collection is completed.

Primary Contact
Courtney Williams
713-440-6070 ext 221
Direct Line: 832-301-3221
cwilliams@apocell.com

Secondary Contact
Apocell Logistics
713-440-6070 ext 122
receiving@apocell.com

For the CPT Vacutainer® blood tube collection:

1. Collect 8 mL of blood into a Vacutainer® CPT tube using a standard method.
2. Note: Do not shake. Vigorous mixing can cause hemolysis.
3. Label the tube with site #, specimen ID, time point (if applicable), ApoCell Project ID Number, and collection date/time. (Please see example below)
   DO NOT REFRIGERATE OR FREEZE.

4. FOLLOWING BLOOD DRAW, THE SPECIMENS WILL BE STORED AT ROOM TEMPERATURE CONDITIONS AT ALL TIMES.

For CellSave® blood tube collection:

1. Preferably, the CellSave® Tube should be the second tube drawn.
2. Collect a minimum of 8 mL of whole blood into the CellSave® tube with 10 mL capacity, which is provided. DO NOT collect sample in EDTA, sodium heparin or ACD tubes.
3. Label each tube with Study Protocol or ApoCell Project ID, sample ID, visit or time point, sample date/time and phlebotomist's initials.
4. Gently invert CellSave® tube 8 times and store at room temperature until it can be picked up. DO NOT REFRIGERATE OR FREEZE.

5. FOLLOWING BLOOD DRAW, THE SPECIMENS WILL BE STORED UPRIGHT, AT ROOM TEMPERATURE CONDITIONS AT ALL TIMES. Prepare the "Specimen Submission" form (see Appendix XX). Notify ApoCell by phone immediately after the samples are collected. An ApoCell-supplied courier will be notified the samples are ready for pickup. The courier will pick up blood specimens within 2 hrs after the blood draw at an designated agreed upon location. Blood will be transported within a secondary temperature-controlled container provided by the courier.

Any residual blood will be discarded. Slides with cells extracted from patient blood will be discarded.

Note: If during the processing phase, any of the tubes break, we will continue to proceed with drawing the other time points and proceed with analyses of the intact tubes (ones already drawn). Also, if the patient does not proceed with surgery (and we are able to get tubes for the 2 time points prior to surgery, these will be analyzed))

APPENDIX F : Specimen Submission Form
Protocol # 2011-1208

Please fill out the information below and submit with specimens:

Protocol:	**Isolation of Circulating Tumor Cells (CTC) from Blood of Pancreatic Cancer Patients by Antibody-Independent ApoStreamTM Technology**					
Apocell Project #:	**AR04**	**Total Specimens:**		**Ship Date:**		
Courier:	**Local**		**Shipping TRK #:**			
Shipping Conditions:	**Ambient Temp**					

Subject Id (Site # - Patient#)	Subject Initials	Specimen Type	Date of Collection	Time of Collection	Cycle	Comments

If submitting more specimens than this space allows for, please attach a roster or spread sheet of specimens.

Specimen(s) prepared by_____ **Date:** _____

ApoCell Contact Information:

Primary Contact: Courtney Williams Main Phone: 713-440-6070 ext 221 Direct Line: 832-301-3221 Email: cwilliams@apocell.comSecondary Contact: ApoCell Logistics 713-440-6070 ext 122 receiving@apocell.com