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Pilot Study Evaluating An Allogeneic GM-CSF-Transduced Pancreatic Tumor Cell Vaccine (GVAX) and Low Dose Cyclophosphamide Integrated with Fractionated Stereotactic Body Radiation Therapy (SBRT) and FOLFIRINOX chemotherapy in Patients with Resected Adenocarcinoma of the Pancreas

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TITLE

Pilot Study Evaluating An Allogeneic GM-CSF-Transduced Pancreatic Tumor Cell Vaccine (GVAX) and Low Dose Cyclophosphamide Integrated with Fractionated Stereotactic Body Radiation Therapy (SBRT) and FOLFIRINOX chemotherapy in Patients with Resected Adenocarcinoma of the Pancreas.

Protocol Synopsis

Title	Pilot Study Evaluating An Allogeneic GM-CSF-Transduced Pancreatic Tumor Cell Vaccine (GVAX) and Low Dose Cyclophosphamide Integrated with Fractionated Stereotactic Body Radiation Therapy (SBRT) and FOLFIRINOX chemotherapy in Patients with Resected Adenocarcinoma of the Pancreas.
Sponsor	Pending
Investigational Product	Whole Cell Vaccine (Allogeneic GM-CSF secreting pancreas vaccine, GVAX).
Objectives	Primary: To evaluate the toxicity of the whole cell vaccine (GVAX) administered along with cyclophosphamide; fractionated SBRT, and FOLFIRINOX. Enrollment is based on traditional 3+3 design with grade 3-4 diarrhea and/or neutropenia defined as the dose limiting toxicity (DLT) within the first 2 cycles (8 weeks) of FOLFIRINOX.
Patient Population	Patients with resected pancreas head, neck, and/or uncinate adenocarcinoma.
	R0 or R1 pancreatic resection
	AJCC stage I-IIB (T1-3, N0-1, M0)
	No evidence of metastatic disease
	ECOG PS 0-1
	Surgery within 10 weeks of study enrollment
	≥ 18 years of age
Pretreatment Requirements	Pathology report available for review
	Operative report available for review
	CT chest/abdomen/pelvis (If allergic to CT scan contrast, obtain abdominal/pelvic MRI with contrast and non-contrast chest CT) showing no disease recurrence
Required Laboratory Tests	Platelets ≥ 100,000/mm^3
	Absolute neutrophil count ≥ 1,000/mm^3
	Total bilirubin ≤ 1.5 x ULN
	Alkaline phosphatase, ALT, and AST ≤ 2.5 x ULN
	Serum Creatinine ≤ 1.5 x ULN
	CA 19-9 ≤ 180 (drawn at least 3 weeks out from surgery)
Study Design	Pilot study SBRT, FOLFIRINOX ± GVAX
	Accrual goal N= 18

Treatment Plan	**Clip placement:** Per the standard of care, all resected patients will have titanium clips placed at the time of surgery outlining the superior and inferior aspect of the retroperitoneal/superior mesenteric artery margin. This will be used to guide SBRT treatment. **Restaging:** Within 3-8 weeks of surgery, patients will be restaged with a history and physical exam, standard bloodwork (including CA 19-9), and a CT of the chest, abdomen, and pelvis (or if IV contrast allergic will have a non-contrast chest CT and abdomen/pelvic MRI). If performance status (ECOG performance status 0-1), incision is healed, drains removed, and no signs of metastatic disease or local recurrence, patients will be considered candidates for the trial (see eligibility section). **Protocol Treatment:** **Cyclophosphamide and Vaccine #1:** Note: **First cohort of patients will not get GVAX or Cy (They will get SBRT and FOLFIRINOX only). (After the first cohort) Vaccine must be initiated within 6-10 weeks from surgery. Patients will receive Cyclophosphamide (Cy) IV over 30 minutes followed by vaccine (GVAX) #1 one day after cyclophosphamide. The dose of Cyclophosphamide will be 200 mg/m^2 intravenously the day prior to each vaccination. Each vaccine will consist of 6 intradermal injections for a total of approximately 5 X 10^8 cells per dose. Each vaccination cycle consisting of cyclophosphamide and vaccine can be referred to as (Cy/Vaccine).
	Stereotactic Body Radiation (SBRT) (6.6 Gy x 5 days) Patients in the cohort NOT receiving vaccine will start SBRT 6-10 weeks post-surgery Patients receiving vaccine will start 13-17 days after Vaccine. SBRT must be started within 12 weeks from the date of surgery. SBRT must be administered at JHU. At least 2 fractions of SBRT will need to be given per week and SBRT must be completed within 2 weeks of initiation.
	FOLFIRINOX: Patients will begin FOLFIRINOX 7-28 days following completion of SBRT. Each patient will receive a total of 6 cycles of FOLFIRINOX.
	Restaging CT or MRI after 3 cycles and following completion of FOLFIRINOX chemotherapy
	Cy/Vaccine #2-5: Cy/Vaccine #2 will be given 35 days (+/- 7 days) after completion of FOLFIRINOX. Cy/Vaccine will be given every 25-31 days for vaccines #3, #4, and #5.
	Continue Cy/Vaccine every 6 months +/- 30 days until progression, toxicity, or subject withdrawal

	(restage prior to each vaccine). As of Amendment #10, boost vaccinations will be administered annually (+/- 30 days).
	Restaging CT (chest/abd/pelvis)or MRI of the abd/pelvis (If IV contrast allergic and non-contrast chest CT) every 6 months following completion of FOLFIRINOX therapy
	Summary (protocol): Surgery (clips placed)→ → **Cy/Vaccine (2 days)** → → SBRT (radiation/5 days) → → FOLFIRINOX (chemotherapy, 24 weeks) → →Cy/Vaccine (once a month for 4 months) → →booster Cy/Vaccine every 6 months (every 12 months as of Amendment #10) until recurrence or stop. ***First 3 enrolled patients will not get Cy/Vaccine.
Duration of Participation	All patients will continue on study treatment until disease recurrence and be followed for overall survival thereafter.
Statistical Rationale	The primary endpoint is safety. The toxicity profile of FOLFIRINOX (F) in the metastatic setting and standard radiation therapy (RT) in the adjuvant setting has been well established. Approximately 20% of patients experience diarrhea, 50% experience non-recoverable neutropenia, and 10% experience thrombocytopenia. We are interested in exploring the combination of FOLFIRINOX with stereotactic body radiation therapy (SBRT) plus GVAX. Toxicity rates of grade 3-4 diarrhea, grade 3 neutropenia, and grade 3-4 thrombocytopenia would be considered unacceptable (dose limiting toxicity-DLT) if they were 40%, 60%, and 40% respectively. Although GVAX is not expected to contribute to these two types of toxicity, we will enroll patients into two cohorts so that only one factor in the therapy is modified at each step. The first cohort will receive FOLFIRINOX plus SBRT in place of standard radiation therapy. The second cohort will receive the same combination plus GVAX. A decision rule similar to the traditional 3+3 design will be used to determine whether or not it is safe to continue on to the next cohort. An initial 3 patients will be treated with F+SBRT. If no toxicities are observed after the first 2 cycles (8 weeks) of adjuvant FOLFIRINOX then the next group of 3 patients will be treated with F+SBRT+GVAX. If 2-3 patients are observed with uncontrolled grade 3-4 diarrhea, 2-3 patients are observed with grade 3-4 thrombocytopenia, or if 3 patients are observed with grade 3-4 neutropenia after the first 2 cycles of FOLFIRINOX then the trial will be halted. Otherwise (i.e. if 1 patient is observed with grade 3-4 diarrhea, 1 patient is observed with thrombocytopenia, or 1-2 patients are observed with neutropenia), then an additional 3 patients will be treated with F+SBRT. If 0-1/6 patients experience grade 3-4 diarrhea, 0-1/6 patients experience grade 3-4 thrombocytopenia, and 0-3/6 experience grade 3-4 neutropenia after 2 cycles of FOLFIRINOX, then the next group of patients will receive F+SBRT+GVAX. Treatment groups for which < 40% experience grade 3-4 diarrhea (i.e. < 2/6), <40% experience grade 3-4 thrombocytopenia (i.e. <2/6), and < 60% experience grade 3-4 neutropenia (i.e. < 4/6) will be considered tolerable. An expansion cohort of 6 patients will be treated with F+SBRT+GVAX, if tolerable, in order to

	refine estimates of toxicity and initial efficacy for a total sample size of 15-18 patients. -Any grade 3-4 toxicity occurring in the first 2 cycles (8 weeks) of FOLFIRINOX and within the context of the decision rule described above will be dose limiting with the exception of 1) nausea, vomiting or diarrhea that improves to ≤ grade 2 with maximal medical management within 5 days; 2) neutropenia that resolves within 5 days; or 3) thrombocytopenia that resolves within 7 days. -Patients who get DLT-qualifying diarrhea but do not adhere to appropriate medical management will be considered inevaluable for DLTs and an additional 3 patients will be enrolled. We hypothesize that the utilization of SBRT instead of standard chemoradiation should result in 1) less immunosuppression 2) less delay in administering full dose chemotherapy (FOLFIRINOX) 3) less toxicity thus improving compliance with subsequent FOLFIRINOX 4) equivalent or improved local control when compared to standard chemoradiation (~20-25% local recurrence).
	Secondary endpoints: 1) To estimate the overall survival (OS), disease-free survival (DFS), freedom from local progression (FFLP), and distant metastases free survival (DMFS) 2) To estimate the association of specific *in vivo* parameters of immune response with time to progression in patients with resected pancreas cancer. The specific immune parameters include vaccine-induced changes in the number, function, avidity, size and diversity of the mesothelin-specific T cell repertoire.

Figure 1: Study Schema

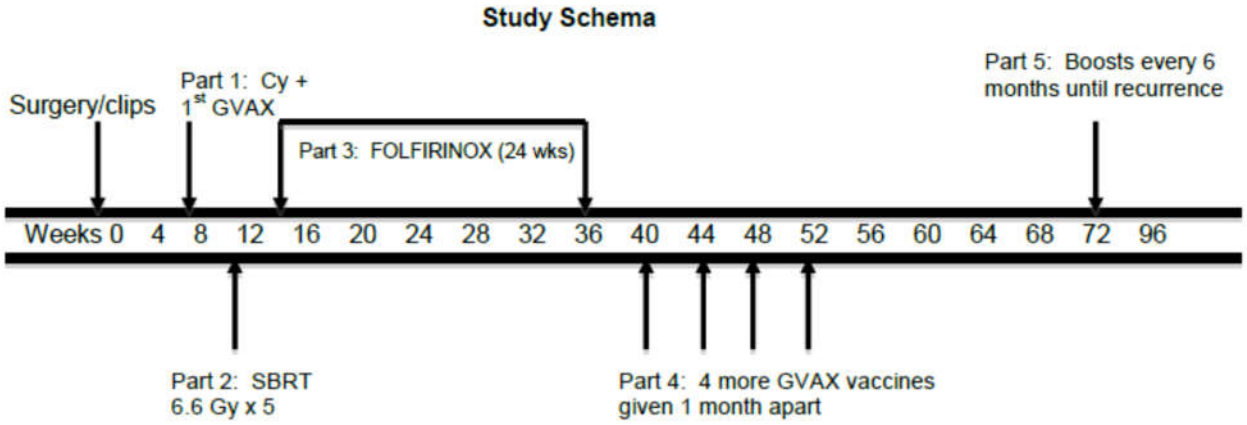

Figure 1: Study Schema. Eligibility will be determined after surgery. All candidates will have clips placed at the time of surgery for SBRT**.** Pre-treatment PBL and serum will be banked 1-2 days prior to each vaccine and 28-32 days after each vaccine. Toxicities will be monitored as part of this study during parts 1, 4, and 5. Safety and efficacy data will be requested and reviewed after SBRT and FOLFIRINOX therapy. Unvaccinated patients will still be asked to donate serum to compare correlates with vaccinated patients. *First 3 patients will not receive GVAX or Cy (FOLFIRINOX and SBRT only).

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1.0 Introduction

1.1 Background

1.1.1 Adjuvant Therapy for Pancreatic Cancer

Pancreas cancer is the tenth leading cause of cancer in the United States with an estimated incidence of 43,140 new cases in 2010(1). The low median survival rates and cure rates for resectable pancreatic cancer imply residual local and systemic microscopic disease that is potentially amenable to adjuvant therapy.

The standard of care of adjuvant therapy has been in flux due to the inconsistent and modest signal seen in trials. Adjuvant chemoradiation has been studied due to the high rate of positive margins and local regional recurrence seen in surgical series. The benefit of 5-fluorouracil based chemoradiation was first seen in a small, randomized trial performed by the Gastrointestinal Tumor Study Group (GITSG)(2). This study showed a striking benefit in median survival and 5-year overall survival compared with observation with the use of post-operative concurrent 5-FU and radiation (40 Gy, split course) followed by 2 years of 5-FU alone in spite of no difference in locoregional control. The EORTC trial seemed to support these findings with a trend towards improved survival with adjuvant chemoradiation alone(3, 4). Additionally, two very large retrospective series, one from Johns Hopkins University (n=616) and one from the Mayo Clinic (n=472), have demonstrated median survival benefits consistent with the GITSG and EORTC studies(5, 6)

The comparative benefit of chemotherapy and chemoradiation was challenged by the European Study Group for Pancreatic Cancer (ESPAC) study, which randomized 541 patients with pancreatic adenocarcinoma to the following four treatment arms under a two-by-two factorial design: a) Observation; b) concomitant chemoradiotherapy alone (20 Gy in 10 fractions over 2 weeks) with 500 mg/m2 5-FU IV bolus during the first three days of radiation therapy(7). The module is repeated after a planned 2 week break followed by no additional chemotherapy; c) chemotherapy alone (leucovorin 20 mg/m2 bolus followed by 5-FU 425 mg/m2 administered for 5 consecutive days repeated every 28 days for 6 cycles); 4) chemoradiotherapy followed by chemotherapy. For the same subset randomized through the original two by two design, chemotherapy alone demonstrated a trend towards improved survival alone (median survival 17.4 months) versus observation alone (15.9 months) but was not statistically significant (p=0.19). The study authors concluded that there was no survival benefit for adjuvant chemoradiotherapy. In addition, the authors concluded that a potential benefit existed for adjuvant chemotherapy alone following surgical resection. Unfortunately the trial's many flaws such as questionable study design and lack of surgical/pathological/radiation quality when compared with institutional outcomes, render the results of this study difficult to interpret. However, ESPAC does highlight the importance of adjuvant chemotherapy.

While the above-mentioned adjuvant studies were being conducted, gemcitabine had emerged as a more effective chemotherapy than 5-FU in the setting of advanced disease(8). Because of this, gemcitabine was evaluated in the post-operative setting. The Radiation Therapy Oncology Group (RTOG) reported on a phase III study of 518 resected pancreatic cancer patients randomized to either 5-FU continuous infusion (250 mg/m2/d for 3 weeks), followed by 5-FU continuous infusion (250 mg/m2/d) during radiation therapy (50.4 Gy in 1.8 Gy/fractions), followed by 2 cycles 5-FU continuous infusion. This was compared to Gemcitabine 1000 mg/m2 weekly X 3, followed by 5-FU continuous infusion during radiation therapy, followed by 3 cycles of Gemcitabine alone(9). While there was a higher incidence of grade 3-4 neutropenia for patients in the

Gemcitabine arm, the median survival was 20 months for the Gemcitabine treated patients versus 16.3 months for 5-FU treated patients (p=.03). In the final manuscript, RTOG reported a survival benefit on multivariable analysis of 20.6 versus 16.9 months (p=.03) in favor of the Gemcitabine chemotherapy arm, restricted to patients with cancer of the pancreatic head. In contrast, the European CONKO-1 study recently published a phase III study of 354 patients randomized to observation or 6 months of Gemcitabine chemotherapy(10). The primary endpoint of this study was DFS with an improvement for the treatment arm (13.4 months v. 6.9 months, p<.001). Further follow-up has shown a survival benefit to chemotherapy.

From these studies, it is evident that a standard adjuvant treatment approach for patients with resected disease has not yet been determined. However, given the above data, gemcitabine or 5-FU based CRT (RTOG 9704) or gemcitabine/bolus 5-FU(CONKO-1/ESPAC-3) can both be viewed as a reasonable standard of care in the adjuvant setting.

1.1.2 Rationale for Cell-Based Immunotherapy of Pancreatic Adenocarcinoma

Immunotherapy is a novel therapeutic approach that has the ability to recruit and activate tumor specific T-cells and induce a cytotoxic response. The potential of this approach is attractive for several reasons: (1) the activation of tumor specific T-cells acts via a mechanism that is distinct from chemotherapy or radiation therapy and would represent a non-cross resistant treatment with an entirely different spectrum of toxicities, (2) the immune system is capable of recognizing a broad diversity of potential antigens while orchestrating selective as well as specific cytotoxic responses.

Rationale for the use of a GM-CSF secreting whole cell vaccine approach

Johns Hopkins University has developed a cytokine secreting tumor vaccine approach that can cure mice of pre-existing tumors. This approach is based on the concept that certain cytokines are required at the site of the tumor to effectively prime cancer-specific immunity. In the only study to directly compare a large number of immune stimulating cytokines, GM-CSF stood out as the most potent cytokine capable of inducing systemic anti-tumor immunity when expressed by the tumor cells for the initial 24-72 hrs of immune priming. GM-CSF is now recognized to be the critical growth and differentiation factor for dendritic cells, the most potent professional antigen presenting cell responsible for priming immune responses against infectious agents and tumor antigens.

Both autologous and allogeneic GM-CSF secreting vaccines have been tested in phase I and II trials in subjects with melanoma, renal cell, prostate, lung, breast and pancreatic cancers. Most of these studies demonstrated evidence of immune activation associated with clinical responses in 10-40% of treated subjects. An allogeneic pancreatic cancer vaccine has completed phase I and II testing at Johns Hopkins University. The results of these studies have been reported and are described below.

While the use of autologous tumor cells may preserve unique antigens expressed by each subject's cancer, the development of an autologous vaccine requires that extensive processing, *in vitro* expansion, and regulatory testing be performed for each individual subject vaccine. These limitations preclude the use of autologous cellular vaccine for most cancers including pancreatic adenocarcinoma. A growing body of evidence supports the immunologic rationale for using allogeneic tumor cells rather than autologous cells as the source of antigen used for the vaccination. First, studies evaluating human melanoma antigens have demonstrated that most of the human tumor antigens identified are shared among at least 50% of known human melanoma tumor cell lines, regardless of whether or not they share the same human leukocyte antigen (HLA) type. In addition, there is now both pre-clinical and human data in pancreatic cancer subjects treated with a GM-

CSF vaccine to support host-derived professional antigen presenting cells (APCs) as the critical cells required to present immunogen to T cells in the context of MHC. Therefore, the vaccine cells do not need to be HLA compatible with the host's immune system as long as they can release cellular proteins (the tumor antigens) for uptake by professional APCs (macrophages and dendritic cells) that are attracted to the vaccine site by GM-CSF. Taken together, the data suggest that relevant tumor antigens can be delivered by an allogeneic tumor and still sufficiently mount an effective immune response.

Two allogeneic cell lines have been developed from neoplastic tissue harvested from the surgical specimens of subjects undergoing pancreaticoduodenectomy at The Johns Hopkins Hospital. These cell lines have been characterized as 100% epithelial by cytokeratin staining. In addition, these cell lines carry the same k-*ras* mutation as the original tumor specimen, which supports the conclusion that these lines are derived from malignant pancreatic tumor cells. The cell lines Panc 10.05 and Panc 6.03 both contain the most common k-ras mutation at codon 12 found in greater than 90% of pancreatic cancer. These lines have undergone extensive regulatory testing and have been shown to maintain GM-CSF secretion, MHC class I levels, cytokeratin positive staining and the original K-ras mutation. These lines also express 2 new immunogenic pancreatic tumor antigens, mesothelin and PSCA. These lines have already been demonstrated to be safe and feasible to produce and administer in a phase I and two phase II studies in both the adjuvant and metastatic setting(11, 12).

1.1.3 Adjuvant studies of the whole cell vaccine (allogeneic GM-CSF secreting pancreatic tumor cell lines)

Results of a Phase I Adjuvant Study at Johns Hopkins

This study was the first clinical trial to test the hypothesis that the allogeneic GM-CSF secreting pancreatic tumor cell lines, hereafter called **whole cell vaccine**, can prime a systemic immune response in subjects with resected pancreatic adenocarcinoma(13). Fourteen subjects with stage II or III disease received an initial vaccination 8 weeks following resection. This was a dose escalation study in which 3 subjects each received 1 X 10^7, 5 X 10^7, and 1 X 10^8 vaccine cells. An additional 5 subjects received 5 X 10^8 vaccine cells. Study subjects were jointly enrolled in an adjuvant chemoradiation protocol for 6 months. Following the completion of adjuvant chemoradiation, subjects were re-assessed and those who were still in remission were treated with 3 additional vaccinations given one month apart at the same original dose that they received for the first vaccination. Toxicities were limited to grade I/II local reactions at the vaccine site, and self-limited systemic rashes, including one documented case of Grover's syndrome. Systemic GM-CSF levels were evaluated as an indirect measure of the longevity of vaccine cells at the immunizing site. As was observed in pre-clinical studies, GM-CSF levels peaked at 48 hours following vaccination. In addition, serum GM- CSF levels could be detected for up to 96 hours following vaccination. These data, together with data from pre-clinical models, would suggest that detectable serum GM-CSF levels may serve as a bio-marker of immune response. The vaccine sites were also evaluated as a measure of the local immune reaction to the vaccine. Eleven of 14 subjects demonstrated a similar local inflammatory response to what has been observed in pre-clinical models and autologous GM-CSF vaccine clinical trials. Post-vaccination DTH responses to autologous tumor cells have been used in previously reported vaccine studies as a surrogate to identify and characterize specific immune responses that are associated with vaccination. In the pancreatic cancer vaccine trial, post-vaccination DTH responses to autologous tumor cells were observed in 1 of 3 subjects receiving 1X 10^8 and in 2 of 5 subjects receiving 5X10^8 vaccine cells. As of December 2010, three long term survivors are still disease-free more than 12 years.

Follow-up phase II study integrating the whole cell vaccine with chemoradiation for resected pancreatic adenocarcinoma

Johns Hopkins has also recently completed a follow-up phase II study of 60 patients with resected pancreatic adenocarcinoma based on the results of their phase I experience(12). The highest dose of vaccine from the phase I study (5 X 10^8 vaccine cells) was used and the adjuvant chemotherapy regimen given in sequence with the vaccine was modified to eliminate mitomycin-C as this drug was thought to depress immune function as measured by a decrease in vaccine induced mesothelin specific T cells following chemoradiation. The common toxicities associated with the vaccine in this study included: local vaccine site skin reactions and systemic rashes similar in severity (grade 1-2) to what was observed in the phase I trial. A full description of toxicities is provided in the Investigator's Brochure. The results from this study include the following:

- The administration of the whole cell vaccine is safe and well-tolerated. Treatment related side effects included transient vaccine injection site reactions.
- Systemic GM-CSF levels were evaluated as an indirect measure of the longevity of vaccine cells at the immunizing site. As was observed in the phase I study, GM-CSF levels peaked at 48 hours following the first and second vaccination but peaked earlier following the 3rd and 4th vaccination with diminution in amplitude. Serum GM-CSF levels following vaccine 5 peaked again at 48 hours and returned to vaccine 1 serum levels. The results would suggest the possibility that the potency of an allogeneic vaccine is diminished with repeated monthly vaccinations, but returns to pre-treatment levels with an extended time interval between boosts.
- As recently reported, (88% node (+), 30% margin (+)) the median survival was 24.8 months (12).
- Post-Immunotherapy induction of mesothelin-specific CD8+ T cells with higher avidity and increased mesothelin epitope recognition (T cell repertoire expansion) correlates with disease free survival (DFS).

The data also support additional boost immunotherapies beyond one year post surgery in future studies.

1.1.4 Rationale for Combining Immune Modulating Doses of Chemotherapy with Vaccine

A large body of murine and human data exists demonstrating that tumors grow despite the simultaneous existence of tumor-specific immune responses. To explain this observation, it has long been hypothesized that subjects with cancer demonstrate peripheral tolerance to their tumor. There is specific data to suggest that immune modulating doses of cyclophosphamide enhances vaccine induced anti-tumor immune responses by inhibiting suppressor T-cell activity. Several clinical trials have since combined cyclophosphamide with vaccination. Recent murine data from our group strongly suggests that cyclophosphamide inhibits the $CD4^+/CD25^+$ regulatory T cells from functionally inactivating the high avidity HER-2/*neu* specific T cell population that is required for effective treatment of mammary tumors(14). These data together with earlier studies provide the rationale to integrate tumor vaccines into current treatment modalities. In addition, since the cyclophosphamide exerts its effect on the regulatory T cells of the immune system rather than on the cancer cell itself, this approach can be applied to treat any type of cancer.

Phase II Trial of whole cell vaccine alone and in sequence with immune modulating doses of cyclophosphamide in subjects with advanced pancreatic cancer

Johns Hopkins University completed a feasibility study of the whole cell vaccine administered alone or in sequence with cyclophosphamide in subjects with advanced pancreatic cancer(11). This study was an open label multi-center study sponsored by Cell Genesys, Inc in collaboration with US Oncology with two cohorts: Cohort A- 30 subjects administered a maximum of six doses of vaccine using two pancreas cancer cell lines each to deliver 2.5 X 10^8 cells intradermally administered at 21 day intervals; Cohort B- 20 subjects administered cyclophosphamide 250 mg/m^2 IV one day prior to vaccine as in Cohort A. The primary objective was to evaluate safety and efficacy of vaccine administered alone or in sequence with cyclophosphamide. Secondary objectives include time to disease progression (TTP) and median overall survival (OS). The following has been reported following to date:

- The administration of the whole cell vaccine either alone or in sequence with cyclophosphamide is safe and tolerated by subjects with advanced pancreatic cancer, the majority of which had received ≥ 2 prior chemotherapy regimens. The median number of vaccines administered was 2 in Cohort A and 3 in Cohort B. Treatment related adverse events reported in > 5% of subjects included local vaccine injection site reactions (100%), fever (14%), rigors (10%) and rash (6%). Grade 3/4 treatment related events identified in only one JHU subject and included leukocytosis, dehydration, and fatigue.
- Serum GM-CSF levels peaked at 48 hours post vaccination consistent with published results in the adjuvant setting and was seen following repeated vaccination, suggesting that vaccine cells are not rapidly cleared by an allogeneic response with repeat administration.
- Stable disease was noted in 16.7 % of subjects in Cohort A and 40% of subjects in Cohort B. Median survival in Cohort A and Cohort B were 2.3 months and 4.7 months respectively in a subject population that had received ≥ 2 prior chemotherapies in 12/20 subjects for Cohort B and in 30/50 subjects overall.

The study represents the first report that the combination of chemotherapy using immunomodulatory doses integrated with a GM-CSF vaccine in subjects with advanced pancreatic cancer is safe and feasible to administer.

*A randomized three-arm neoadjuvant and adjuvant feasibility and toxicity study of a GM-CSF secreting allogeneic pancreatic cancer vaccine administered either alone or in combination with either a single intravenous dose or daily metronomic oral doses of cyclophosphamide for the treatment of patients with surgically resected adenocarcinoma of the pancreas***.**

We are currently conducting a neo-adjuvant and adjuvant vaccine study comparing 2 methods of Cyclophosphamide administration given with vaccine versus vaccine alone. At present, this study is recruiting 39 research participants, who are randomized into three arms. All participants are receiving the first vaccination of 5 x 10^8 cells of an equal mixture of two allogeneic GM-CSF secreting pancreatic vaccine cell lines (Panc 10.05 and Panc 6.03) two weeks before a pancreaticoduodenectomy, a second vaccination between 6 and 10 weeks following the pancreaticoduodenectomy (4 weeks prior to adjuvant chemoradiation), and then four additional vaccinations once every 28-days beginning 1-2 months following completion of chemoradiation. A total of six prime vaccinations are administrated. For participants in Arm B and Arm C, each vaccination is combined with a single low-dose cyclophosphamide or repetitive twice-daily metronomic doses of cyclophosphamide, respectively. The vaccine dose is the same dose found to be safe and to induce immune responses in the above described phase I and II studies. Since approval in July 2008, we have enrolled and treated 46 patients (between 7/30/08 and 08/31/11). No serious adverse events were observed in these patients as a result of vaccine therapy with or without cyclophosphamide. Similar to prior vaccine studies, all

subjects experienced grade I/II local reactions at the vaccine site. Systemic reactions rarely occurred and were self-limiting. Systemic lymphopenia, which is anticipated to result from the cyclophosphamide treatment, occurred in two patients and was resolved within one week and prior to the surgery. No patients' surgery was delayed because of the neoadjuvant treatment. No additional adverse events were observed with repeated dosing of cyclophosphamide prior to each vaccine. Thus, the preliminary results suggest that the PDA vaccine and/or immune modulating doses of cyclophosphamide is safe and feasible to give prior to surgical resection of PDA and in a repeated dosing schedule.

1.1.5 Rationale for replacing standard chemoradiation with fractionated stereotactic body radiation therapy (SBRT) in the adjuvant setting.

Standard chemoradiation (CRT) requires 5-6 weeks of therapy and often attenuated doses of chemotherapy when compared to adjuvant regimens that use full doses of chemotherapy (CONKO, RTOG 9704)(9). Standard CRT causes more toxicity (acute and chronic) when compared to chemotherapy alone and less compliance of subsequent maintenance chemotherapy(15). Therefore, many investigators have either eliminated radiation from adjuvant therapy (CONKO/ESPAC-3) or delayed giving radiation until adjuvant full-dose chemotherapy has been completed(10). Although eliminating or delaying radiation therapy may decrease the risk of metastatic disease, it may also increase the risk of local recurrence, which can cause extensive morbidity (pain and obstruction) and result in death(16). In our phase I/II adjuvant vaccine trials, we also found that adjuvant CRT with or without mitomycin-C caused immunosuppression and a decrease in the vaccine-mediated immune response. Therefore we propose utilization of fractionated stereotactic radiation therapy to the tumor bed plus a small margin (1 cm) followed by FOLFIRINOX chemotherapy as an alternative to standard CRT. The most common site of a localized recurrence is the tumor bed or superior mesenteric artery (SMA) margin. Therefore, we recommend treating only the SMA region (plus a small margin) in this study.

1.1.6 Rationale and safety data for stereotactic body radiation therapy

Koong *et al.* previously used the Cyberknife™ stereotactic radiosurgery system to demonstrate that a **single dose** of 25 Gy stereotactic body radiotherapy (SBRT) was feasible to administer in patients with locally advanced pancreatic cancer(17). Furthermore, this dose of SBRT resulted in near 100% progression free survival and effectively palliated symptoms related to the local growth of pancreatic tumors. Based upon this study, they also completed a phase II study assessing the efficacy of combining a standard five-week course of chemoradiotherapy followed by a stereotactic radiosurgery boost to the primary tumor in patients with locally advanced pancreatic cancer(18). In this cohort of 19 patients, 100% of tumors were without local progression. However, all patients eventually developed metastases with a median time to progression of 5.5 weeks.

More recently, another phase II study treated locally advanced pancreatic cancer patients with gemcitabine followed by 25 Gy of SBRT delivered with Cyberknife and maintenance gemcitabine chemotherapy. In this study, the excellent progression free survival was confirmed from previous studies (81%). The median overall survival was 11.4 months, median time to progression was 9.7 months and the 1-year survival was 50%(19). There were no significant acute GI toxicities, however, of the 15 patients alive >6 months after SBRT, 7 (47%) experienced Grade 2 or greater GI toxicity, with 2 (13%) of the 15 experiencing Grade 3 or greater GI toxicity.

Trilogy™ and Synergy™ are commercially available (Varian Medical Systems, Palo Alto CA and Elekta AB, Stockholm, Sweden, respectively) linear accelerators specifically designed for image guided radiation therapy (IGRT). These machines combine a conventional high-energy linear accelerator with a kV imager capable of volumetric, cone beam CT (CBCT). Because of these innovations, it is possible to deliver highly accurate, stereotactic radiation treatments. Trilogy™ and Synergy™ eliminate a large proportion of the uncertainties in tumor location.

A recent protocol evaluated full dose gemcitabine before and after single fraction SBRT delivered with the Trilogy™ linear accelerator(20). Preliminary results indicate that the local progression free survival was comparable to what was previously observed (90%) with CyberKnife treatment. All acute toxicity was grade 2 or less, however a minority of patients developed late duodenal ulcers (15%), including 1 perforation (5%) with a single fraction of SBRT.

The mortality rate for pancreatic cancer approaches 100%. Current therapies provide only partial palliation of symptoms and slight prolongation of survival. Better treatment is clearly needed. Stereotactic radiation therapy may more effectively ablate pancreatic tumor cells when compared to conventional radiation therapy, and translate into improved patient survival with decreased side effects. To date, Stanford has treated more than 150 patients with SBRT and this treatment has resulted in local control rates of >90% with acceptable acute GI toxicity. We predict that SBRT will not adversely impact patients' quality of life, however this has not been thoroughly studied in the adjuvant setting. The majority of patients with locally advanced pancreatic cancer treated with SBRT had a clinical benefit as assessed by decreased pain, decreased fatigue, and increased weight. A single fraction of SBRT (25 Gy x 1) has resulted in excellent tumor control. However, close to 50% of these patients developed late duodenal toxicity within one year primarily because of its proximity to the pancreas. At this time there is no clear consensus regarding an optimal SBRT fractionation schedule for unresectable pancreas cancer(20).

In June 2010, we opened a multi-institutional study (Johns Hopkins, Stanford, and Memorial Sloan Kettering) where we are treating locally advanced pancreatic cancer patients with SBRT for 6.6 Gy x 5 fractions. In this study we are treating the tumor plus a 2 mm margin expansion. The small margins are possible due to daily imaging, markers to guide therapy, and decreasing breathing motion with airway breathing control (ABC). This fractionation schedule is based upon personal conversations with Dr. Timmerman who has conducted extensive research on SBRT. Based on his recent study, this fractionation schedule is predicted to provide equivalent tumor control probability as 25 Gy x 1 while resulting in less normal tissue toxicity(21). To date, we have treated 35 patients, all with >4 months of follow-up and there have not been any reported grade 3 or 4 toxicities.

While a majority of studies have evaluated SBRT in the locally advanced setting, one study by Hong et al evaluated 5 Gy x 5 in the neoadjuvant setting. In his study patients received SBRT delivered with protons and concurrent 5-FU by treating the tumor and adjacent peri-pancreatic lymph nodes. Thus far, no patients have experienced grade 3/4 acute or chronic toxicity (38).

There is one study reporting on the role of SBRT in the adjuvant setting(22). In this study, 24 pancreatic cancer patients with close or positive margins were treated with a single fraction of adjuvant SBRT (20-30 Gy/1 fraction) following surgical resection. The median target volume was 11 cc (4.5-30 cc). Eighteen patients were treated with the Cyberknife(R) Robotic Radiosurgery System and six patients were treated with Trilogy intensity-modulated radiosurgery. The median follow-up for all patients was 12.5 months

(1.4-39.5 months), and among surviving patients it was 16.3 months (2-39.5 months). The Freedom from local progression (FFLP) rates at 6 months, 1 and 2 years were 94.7%, 66%, and 44%, respectively. Overall, FFLP was achieved in seven (87.5%) patients with close margins, and 10 (62.5%) with positive margins. After SBRT, 19 patients resumed or started a 6-month course of gemcitabine-based chemotherapy at a median interval of 18 days (range, 9-31 days) post-SBRT. The median OS was 26.7 months and the 1- and 2-year OS rates were 80.4% and 57.2%, respectively. Of the 24 patients, 12 (50%) developed distant metastases of whom two (25%) had close margins and 10 (62.5%) had positive margins. Ten patients (41.7%) were free of progression at last follow-up (range, 3-39.5 months). Three patients (12.5%) had grade 1-2 acute GI toxicities, and two patients (8.3%) had grade 1 and 2 late toxicities. No patients experienced grade 3 or 4 toxicity, including bowel perforation, secondary to SBRT. While these rates of recurrence are higher than historical controls, it is important to note that this study only included patients with close or positive margins. Based on these results, we propose treating the tumor bed (based on surgical clips) plus a 1 cm expansion to a total of 33 Gy in 5 fractions (6.6 Gy/fraction).

1.1.7 Utilization of FOLFIRINOX in the adjuvant setting

The use of FOLFIRINOX (5-FU, leucovorin, Irinotican, and oxaliplatin) in the adjuvant setting has not yet been reported in the literature, however several academic centers have started integrating FOLFIRINOX as their standard of care and it appears tolerable (Hong, Mass General Hospital-personal communication). Although, FOLFIRINOX has not yet been validated in the adjuvant setting, Conroy et al. have demonstrated an impressive survival benefit over gemcitabine in a recently reported phase III study. Given the dismal survival of gemcitabine in the adjuvant setting, utilization of FOLFIRINOX in the adjuvant setting may result in a similar survival benefit (months) seen in patients with metastatic disease. However, we believe this should be evaluated in a prospective clinical trial. Further, it may be difficult to combine FOLFIRINOX with standard chemoradiation (6 weeks) and therefore may be better tolerated with a short course of SBRT as outlined above.

1.1.8 Rationale for the current study

Given the modest results and lack of progress made with conventional cytotoxic chemotherapeutics and FDA-approved biologic agents, it is clear that a paradigm shift is needed. Our phase II study of the whole cell vaccine strategy (GVAX) has been completed with provocative results. Further analysis suggests that a benefit may be more durable if booster doses of the vaccine are given beyond 6 months. There is reason to believe that the addition of immunomodulating doses of cyclophosphamide will enhance the efficacy of the vaccine. It is likely that a short course of fractionated stereotactic body radiation therapy (SBRT) delivered to the tumor bed plus a small margin will cause less immunosuppression than standard adjuvant chemoradiation with equivalent local control rates as standard chemoradiation. Also, by including FOLFIRINOX in the adjuvant setting, we expect similar survival improvements to what has been seen in patients with metastatic disease. This study is also an opportunity to further assess whether the immune correlates found promising in our previous study are similar when chemotherapy (FOLFIRINOX) and radiation (SBRT) are altered.

1.1.9 Study position nationally

We expect to observe an improved median DFS and OS with this combination of fractionated SBRT/FOLFIRINOX integrated with or without GVAX + Cy. Both the FOLFIRINOX and vaccine therapies have been tested in PDA patients with some improvement over other therapeutic options. We anticipate the fractionated SBRT will result in similar local control to standard therapy while allowing more rapid integration of systemic therapies with less immunosuppression. We are also improving on the

vaccine by adding low dose Cy to each vaccine cycle to deplete Tregs which have been shown to hinder vaccine induced immune responses. This concept of adding the pancreatic vaccine to a short course of fractionated SBRT and aggressive adjuvant chemotherapy (FOLFIRINOX) represents a natural step forward in adjuvant treatment. The role of stereotactic therapy has been adopted as the standard of care for some primary tumors including early stage lung cancer. While a single fraction of SBRT has shown promise in unresectable PDA, fractionated SBRT (5 versus 1 treatment) in the adjuvant setting may result in similar tumor control with less side effects than standard adjuvant CRT. Results of gemcitabine in combination with other agents in the metastatic setting have been negative (5-FU, oxaliplatin, capecitabine, bevacizumab, cetuximab) or modestly positive (erlotinib)(25-28). A recent publication reported encouraging results of FOLFIRINOX in patients with metastatic disease when compared to gemcitabine alone, although a majority of patients had pancreatic tail lesions and patients experienced a high rate of grade 3-4 toxicity requiring significant dose reductions and hospitalizations stressing the need for this to be evaluated in a prospective adjuvant clinical trial(29). This proposed strategy marks a significant departure in paradigm from these other strategies and is backed by strong institutional evidence as well as scientific rationale. We also expect to be able to estimate the frequency of the toxicities that were initially identified in the phase I and II studies and that are vaccine related. Based on these studies, we do not expect to detect significant local or systemic toxicities that are different in a multi-institutional setting from what we have already observed since we expect that the spectrum of toxicities from the SBRT are different from the vaccine and there should be no interactions. The toxicities of low-dose Cy have also been well characterized. The toxicity profile of the combination of low-dose Cy and vaccination can be estimated from the above-described phase II study in patients with metastatic PDA and our experience in the neo-adjuvant/adjuvant setting. Because PDA has a very poor prognosis and there are few available treatment options, a <20% non-life threatening toxicity rate would be considered acceptable in this study (NCI Common Toxicity Criteria grade 3 or less). Based on the low toxicity profiles of vaccine and low-dose Cy, administration of preoperative vaccine/Cy is not anticipated to change the outcome of this regimen.

1.2 Inclusion of Women and Minorities

We expect to recruit, enroll, and treat about 18 patients in this study. No patient will be excluded from the study on the basis of sex, race, ethnic background, or socio-economic status. Based on pancreatic cancer epidemiologic information as well as data from the Johns Hopkins Hospital we believe the sex distribution will be approximately 10 males and 8 females for a total of 18 patients in this study. The average age will be between 35 and 75 with a median age of approximately 58-62.

The Johns Hopkins Hospital is located in East Baltimore, a historically medically underserved area of the city. Part of the mission of the Hospital is to provide medical care and access to innovative research to the surrounding community. The Sidney Kimmel Comprehensive Cancer Center also serves as a regional referral center for the mid-Atlantic region. Therefore, we anticipate the study population to reflect the patients that Johns Hopkins serves, and target the enrollment of individuals by ethnicity and race as indicated in the attached Targeted/Planned Enrollment Table.

Despite the mission of the Hospital to serve the surrounding community, the enrollment of African American individuals in clinical trials at the Sidney Kimmel Comprehensive Cancer Center remains lower than it should be. Every effort will be made to recruit and include underserved individuals and racial minorities in this study. To accomplish this, we will take advantage of two programs active at Johns Hopkins. First, we will utilize outreach programs developed by the School of Public Health to make the local community aware of the opportunity to participate in clinical research. This program reaches out to the surrounding African American community through the local churches and religious

network. Second, we will take advantage of the Hopkins/Howard partnership. Howard University is a historically black institution that serves the city of Washington DC, and has a large community of African Americans as a referral base. There is no racial, ethnic, or economically disadvantaged group of individuals that is excluded from study participation.

2.0 Objectives

2.1 Primary Objective

To evaluate the toxicity of the whole cell vaccine (GVAX) administered along with cyclophosphamide; fractionated SBRT, and FOLFIRINOX. Enrollment is based on traditional 3+3 design with grade 3-4 diarrhea and/or neutropenia defined as the dose limiting toxicity (DLT) within the first 2 cycles (8 weeks) of FOLFIRINOX.

2.2 Secondary Objective(s)

2.2.1 To estimate the overall survival (OS), disease-free survival (DFS), freedom from local progression (FFLP), and distant metastases free survival (DMFS)

2.2.2 To estimate the association of specific *in vivo* parameters of immune response with time to progression in patients with resected pancreas cancer. The specific immune parameters include vaccine-induced changes in the number, function, avidity, size and diversity of the mesothelin-specific T cell repertoire.

3.0 On-Study Guidelines

This clinical trial can fulfill its objectives only if patients appropriate for this trial are enrolled. All relevant medical and other considerations should be taken into account when deciding whether this protocol is appropriate for a particular patient. Physicians should consider the risks and benefits of any therapy, and therefore only enroll patients for whom this treatment is appropriate.

4.0 Eligibility Criteria

4.1 Documentation of Disease:

4.1.1 Histologic Documentation: Histologic evidence of invasive ductal adenocarcinoma. Patients with mixed adenocarcinoma tumors are allowed if the predominant component is adenocarcinoma. Adenocarcinoma (invasive component) arising from intraductal pancreatic mucinous neoplasm (IPMN) is permitted.

4.1.2 Stage: Completely resected with a pancreaticoduodenectomy and an R0 (microscopically negative margins) or R1 (microscopically positive margins, AJCC stage I-IIB, T1-3, N0-1, M0.

4.1.3 Tumor Site: Pancreas head, neck, and/or uncinate process.

4.1.4 Block/slides request: It is recommended that patients have paraffin-embedded tissue to confirm a diagnosis of adenocarcinoma.

4.2 Prior Treatment

4.2.1 No prior systemic therapy or radiation therapy for pancreatic cancer.

4.2.2 Enrollment must occur within 10 weeks from surgical resection.

4.2.3 Titanium clips (minimum 1) will be placed to delineate the retroperitoneal/SMA margin at the time of surgery for SBRT treatment planning.

4.2.4 Prior adjuvant or neoadjuvant therapy is (not) allowed.

4.3 Patients must not have evidence of recurrent or metastatic disease on post-operative re-staging CT scans of the chest/abdomen/pelvis with IV contrast or if IV contrast allergic, MRI abdomen/pelvis with contrast and non-contrast chest CT.

4.4 No prior abdominal radiation.

4.5 Age ≥ 18 years of age.

4.6 ECOG Performance Status 0 or 1.

4.7 Pregnancy/Nursing Status- Patients must not be pregnant or nursing. Women of childbearing potential must have a negative pregnancy test within 1 week of enrollment and agree to birth control.

4.8 Required Initial Laboratory Values:

Platelets	≥ 100,000/mm^3
Absolute neutrophil count	≥ 1,000/mm^3
Total bilirubin	≤ 1.5 x ULN
Alkaline phosphatase	≤ 2.5 x ULN
ALT	≤ 2.5 x ULN
AST	≤ 2.5 x ULN
Serum Creatinine	≤ 1.5 x ULN
CA 19-9	≤ 180, drawn at least 4 weeks out from surgery

4.9 Exclusion criteria

4.9.1 Patients in whom histologic diagnosis is not consistent with ductal adenocarcinoma such as pure squamous cell, colloid, islet cell, serous or mucinous cystadenoma or cystadenocarcinoma, carcinoid, small or large cell carcinoma, intraductal oncocytic papillary neoplasms (IOPN) osteoclast-like giant cell tumors, acinar cell carcinoma, pancreatoblastoma, solid pseudopapillary tumors, undifferentiated small cell carcinoma, non-epithelial tumors (sarcoma, GI stromal tumor, lymphoma). In addition, patients with mixed adenocarcinoma tumors will not be included if the predominant invasive component of the tumor is not ductal adenocarcinoma.

4.9.2 Tumors of the pancreatic body/tail.

4.9.3 Known history of HIV or active hepatitis B or C infection.

4.9.4 Adenocarcinoma originating in the ampulla, distal bile duct, or duodenum.

4.9.5 Patients with gross residual tumor (R2 resection) following surgical resection.

4.9.6 Patients with recurrent disease.

4.9.7 Metastatic disease including peritoneal implants, liver and/or lung involvement.

4.9.8 Pregnant or lactating women: Women of childbearing potential must have a negative pregnancy test within 1 week prior to study treatment. (Contraception Warning: Women of childbearing potential and men enrolled in the study must agree to practice an effective method of birth control during the study and for at least four weeks after their last treatment on protocol.)

4.9.9 Presence of existing second malignancies except for non-melanoma skin cancer or a history of second malignancies in the past five years.

4.9.10 Uncontrolled medical conditions that could potentially increase the risk of toxicities or complications of the vaccine, especially heart disease. If the patient has a history of heart disease, they should be evaluated by a cardiologist prior to enrollment.

4.9.11 Active infections.

4.9.12 Active autoimmune diseases or history of autoimmune disease requiring chronic medical treatment with systemic immunosuppressants including: inflammatory bowel disease, systemic vasculitis, scleroderma, psoriasis, multiple sclerosis, hemolytic anemia or immune thrombocytopenia, rheumatoid arthritis, SLE, and Sjogren's syndrome, sarcoidosis. Asthma or COPD that does not require systemic corticosteroids or routine use of inhaled steroids is acceptable.

4.9.13 Chemotherapy, radiation therapy or biologic therapy within 28 days prior to administration of vaccine.

4.9.14 Participation in an investigational new drug trial within one month prior to administration of vaccine.

4.9.15 Systemic steroids within 28 days prior to receiving the whole cell vaccine.

4.9.16 Major active medical or psychosocial problems that could be exacerbated by this treatment.

4.9.17 Inability to begin protocol treatment within 70 days (10 weeks) after surgery or the development of postoperative complications which require ongoing medical care past week eleven that would significantly delay time to adjuvant therapy. Patients should be consuming oral nutrition and maintaining weight, have no ongoing requirement for biliary stenting, nor have persistence of a significant wound infection.

4.9.18 Known or suspected hypersensitivity to GM-CSF, pentastarch, hetastarch, corn, DMSO, fetal bovine serum, or trypsin (porcine origin)

4.9.19 Age >76

4.9.20 History of cardiac ischemia or cardiovascular disease, the patient must first be cleared by their cardiologist.

5.0 Enrollment Requirements

5.1 Informed Consent**: the patient must be aware of the neoplastic nature of his/her disease and consent must be provided after being informed of the procedure to be followed, the experimental nature of the therapy, alternatives, potential benefits, side-effects, risks, and discomforts. Human protection committee approval of this protocol and a consent form is required.**

5.2 HIPAA requirements

6.0 DATA SUBMISSION AND MODALITY REVIEW

6.1 Surgical Protocol Quality Assurance Requirements

While all patients will have had surgery as required by eligibility, surgery is not part of the treatment protocol. Therefore, there will not be specific surgical quality assurance required. However, operative notes will be required and must describe a pancreatic surgery performed with intent of removing all gross disease and clips must be placed outlining the superior mesenteric artery margin.

6.2 Pathologic Protocol Quality Assurance Requirements

For each patient, the pathological quality assurance form (Oncospace database) must be completed. This will document gross tumor size, AJCC T-stage, differentiation, presence

of lymphovascular invasion (LVI) and perineural invasion (PNI), number of lymph nodes assessed, number of lymph nodes involved with tumor, and margin status of the uncinate, retroperitoneal, pancreatic transection, and biliary transection margin (positive, close defined as $\leq$ 1 mm, negative defined as > 1 mm, or not assessed).

6.3 Radiation Quality Assurance Requirements

6.3.1 QA Documentation

Prior to the start of radiation therapy, the following information will be recorded:

6.3.2 Treatment Planning System Output

- Digitally reconstructed radiographs (DRR) or simulation films for each treatment field and orthogonal (anterior/posterior and lateral) images for isocenter localization for each group of concurrently treated beams.
- Isodose distributions (in absolute dose) for the composite SBRT treatment plan in the axial, sagittal and coronal planes at the center of the treatment or planning target volume. The planning target volume, isocenter and the normalization method (if normalized isodoses are sent) must be clearly indicated.
- Dose volume histograms (DVH) for the composite treatment plan for all target volumes and required organs at risk.
- Treatment planning system summary report that includes the monitor unit calculations, beam parameters, calculation algorithm, and volume of interest dose statistics.

7.0 Required Data

7.1 Screening visit

All screening evaluations must be completed within 28 days prior to study enrollment.

Pre-Study Testing Intervals

To be completed within **28 days** before study drug administration:

- Complete history, including review of medications.
- Physical examination, including height, weight, vital signs (blood pressure, temperature, pulse, respiratory rate), ECOG performance status.
- All laboratory blood tests, including pregnancy test.
- CT scan chest, abdomen, pelvis with IV contrast or if IV contrast allergic, MRI abdomen/pelvis with contrast and non-contrast chest CT. Pathology report and operative report available for review by the Principal Investigator.

To be collected before the first vaccination:

- All specimens for correlative studies need to be collected as in **Section 7.1.1** below and banked for analysis of the secondary immune endpoints. This includes blood samples for HLA testing, monitoring of antigen-specific T cell responses and antibody responses.

Table 1: Pre-Treatment, Cyclophosphamide (Cy)/Vaccine (GVAX) #1

	Screening (D-28 to D0)	D0: Cy	D1: Vaccine #1
Tests & Observations			
History	X	X	
Physical exam	X	X[1]	
Vital Signs	X	X	X[2]
Height	X		
Weight, BSA[4]	X		
Performance Status	X	X	
Toxicity Assessment			X
Central Pathology Review	X		
Operative Report Review	X		
Vaccine Site Assessment[3]			X
Treatment (patients 4-18 only)			
Cyclophosphamide		X[4]	
Vaccine			X[4]
Laboratory Tests			
Clinical hematology[5]	X	X[6]	
Serum chemistry[5]	X	X[6]	
Urinalysis	X		
Pregnancy Test[7]	X		
CA 19-9	X		
Staging & Scans			
CT chest/abdomen/pelvis with contrast (MRI abdomen/pelvis with contrast and non-contrast chest CT if IV contrast allergy)	X		
Correlative Tests (For patients who consent to correlative studies)			
HLA typing (10 ml)	X		
Immune Monitoring (up to 180 cc)	X		
Serum banking (up to 20 cc)	X		

[1]Problem oriented exam
[2]Vital signs will be taken prior to and after the 30 minute observation period.
[3]Vaccine site reactions will be assessed and documented until resolution.
[4]First 3 patients will not receive Cy/GVAX and do not require BSA measurement at screening.
[5]Clinical hematology: CBC with differential, platelet count, ANC, ALC, and AEC;
serum chemistry: sodium, potassium, chloride, bicarbonate, glucose, BUN, creatinine, ALT, AST, alkaline phosphatase, bilirubin, total protein, albumin, and calcium.
[6]Laboratory tests can be drawn up to 1 week prior to Cyclophosphamide administration.
[7]For women of childbearing potential only, obtained within 7 days.

Table 2: SBRT Treatment

	SBRT
Tests & Observations	
History	X[1]
Physical exam	X [1]
Vital Signs	X [1]
Weight	X [1]
Performance Status	X[1]
Toxicity Assessment	X[1,2]

[1]Day 1 and 5 of SBRT treatment
[2]Within 7 days of completion of SBRT. Toxicity Assessment obtained by study clinician or study nurse contact with the research participant, his/her family, or health care providers.

Table 3: FOLFIRINOX
(Per institution guidelines, can be administered locally)

	FOLFIRINOX (24 Weeks)
Tests & Observations	
History[5]	Pre dose 1 of each Cycle
Physical exam[1,5]	Pre dose 1 of each Cycle
Vital Signs[5]	Pre dose 1 of each Cycle
Weight, BSA[5]	Pre dose 1 of each Cycle
Performance Status[5]	Pre dose 1 of each Cycle
Toxicity Assessment[5]	Pre dose 1 of each Cycle
Laboratory Tests	
Clinical hematology[2,3,4,5]	Within 72 hrs of day 1 and day 15 of each cycle
Serum chemistry[2, 3, 4,5]	Within 72 hrs of day 1 and day 15 of each cycle
PT/INR[5]	As needed
CA 19-9	Pre FOLFIRINOX, after 3 cycles, and post FOLFIRINOX
Correlateive Tests	
Immune Monitoring (up to 180 cc)	Within 7 days of Cycle 1 Day 1
Serum banking (up to 20 cc)	Within 7 days of Cycle 1 Day 1
Staging & Scans	
CT chest/abdomen/pelvis or MRI abdomen/pelvis (either with contrast)	After 3 cycles (12 weeks) and after completion.

[1]Problem oriented exam for cycles 2-6
[2]Recomended prior to each cycle per institutional guidelines with the final decision based on judgment of treating oncologist.
[3]See additional recommended schedule for associated toxicities with the final decision based on the judgment of the treating oncologist.
[4]Clinical hematology: CBC with differential, platelet count, ANC, ALC, and AEC; serum chemistry: sodium, potassium, chloride, bicarbonate, glucose, BUN, creatinine, ALT, AST, alkaline phosphatase, bilirubin, total protein, albumin, and calcium.
[5]Not collected during cycles 4-6 if patient is having FOLFIRINOX treatment locally.

The first 3 patients will be evaluated after their first 2 cycles of FOLFIRINOX at JHU The number, type, and degree of toxicities throughout follow-up will be tabulated. Patients in the cohort receiving Cy/GVAX may opt to have cycles 4-6 of FOLFIRINOX from their local oncologist. Efforts will be made to collect any CA19-9, CT scans, and toxicity data upon patient's return for their post-FFX/pre-Cy/GVAX #2 visit. See section 15.3 regarding special consideration for AE recording and reporting during local FOLFIRINOX treatment.

Table 4: Cyclophosphamide (Cy)/Vaccine #2 & Beyond; Longer-Term Follow-Up or Off Study

	Cyclophosphamide & Vaccine #2-5 (q 28 days, +/- 3 days)		**Cyclophosphamide & Vaccine 6+ (q 12 months,+/- 30 days)**		**Off Study Evaluation[6]**
	D0: Cy	**D1: Vaccine**	**D0: Cy**	**D1: Vaccine**	
Tests & Observations					
History[1]	X		X		X
Physical exam[1]	X		X		X
Vital Signs	X	X[2]	X	X[2]	X
Weight, BSA	X		X		
Performance Status[1]	X		X		X
Toxicity Assessment	X	X	X	X	X
Vaccine Site Assessment[3]	X		X		X
Treatment					
Cyclophosphamide	X		X		
Vaccine		X		X	
Laboratory Tests					
Clinical hematology[1, 4]	X		X		X
Serum chemistry[1, 4]	X		X		X
CA 19-9[1]	X		X		X
Staging & Scans					
CT chest/abdomen/pelvis with contrast or MRI abdomen/pelvis with contrast and non-contrast chest CT, if IV contrast allergy	X (To be done within 7 days prior to Cy/Vaccine #2 and 5 only i.e., q 3 months)		X (To be done within 28 days prior to Cy/Vaccine) until progression or death		Defer to treating physician
Correlative Tests					
Immune Monitoring (up to 180 cc)	X[5]		X[5]		X[7]
Serum Banking (up to 20 cc)	X[5]		X[5]		X[7]

[1]Laboratory tests, history, physical exam, and ECOG can be completed up to 1 week prior to Cyclophosphamide administration #2-5, or up to 28 days prior to Cyclophosphamide administration #6 and beyond.

[2]Vital signs will be taken prior to and after the 30 minutes observation period following each vaccine

[3]Vaccine site reactions will be assessed and documented until resolution

[4]Clinical hematology: CBC with differential, platelet count, ANC, ALC, and AEC; serum chemistry: sodium, potassium, chloride, bicarbonate, glucose, BUN, creatinine, ALT, AST, alkaline phosphatase, bilirubin, total protein, albumin, and calcium.

[5]For vaccine #5 and beyond, immune monitoring and serum banking specimens will be also collected on day 28 (+/- 14 days). As of Amendment #9, research bloods are no longer required to be collected.

[6]Recomended, but not required

[7]As of Amendment #9, research bloods are no longer required to be collected.

7.1.1 Research Blood Collection and Submission:

One 10 ml yellow top tube (Catalog # BD-364606) collected prior to administration of the first dose of Cyclophosphamide-vaccine is required for HLA-typing. Up to 180 cc blood in 10 ml lavender/purple top tubes (Catalog # BD-366643) or 180cc heparinized syringes and up to 20 cc blood in 10 ml serum separator tubes (SST) (Catalog # BD-366430) collected at the indicated time points are required for immune monitoring and serum banking respectively. As of Amendment #9, research bloods are no longer required to be collected.

Procedure for Isolation of Serum

1) Serum separator tubes (SST) should be spun down within 1 hour after blood draw for best results. The blood in the SST should look deep red in color and thick in appearance.

2) Blood is to be at room temperature (18°C - 25°C); hold the centrifuge at room temperature prior to processing.

3) Invert SST 5-10 times immediately prior to spinning.

4) Place SST in the centrifuge and balance the rotor.

5) Spin SST in centrifuge at 2500 rpm for 10 minutes in swinging bucket (SW) or 15 minutes in a fixed angle (FA) rotor at room temperature (18°C - 25°C). Make sure that the centrifuge reaches speed and is maintained throughout the entire spin.

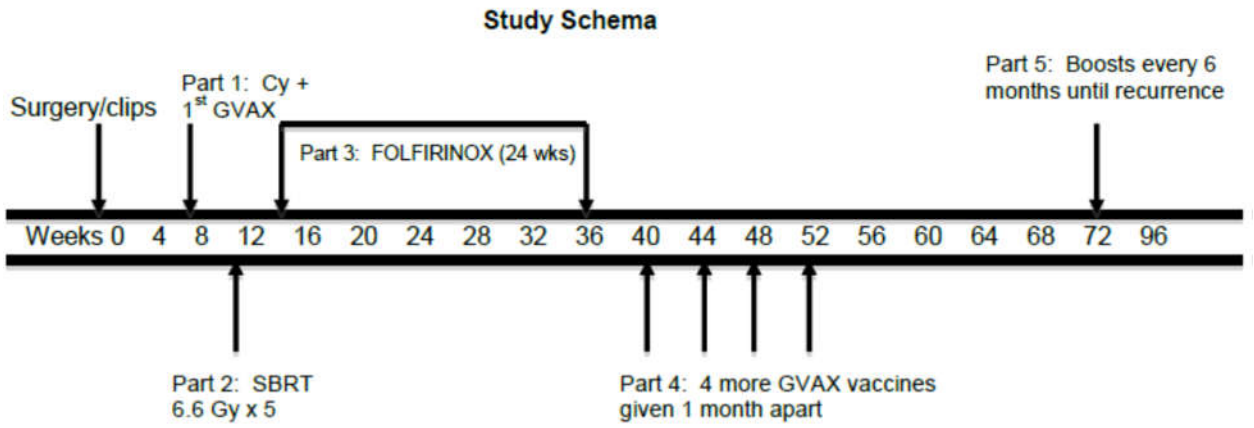
6) Carefully remove SST from centrifuge and visually inspect it for proper separation. If separation is not observed, repeat the spin one more time. Do not repeat more than once. If separation does not occur following the second spin, send it in its unseparated form.

7) Place SST in FedEx Clinical Pak and mark date and time it was spun down.

8.0 Treatment Plan

Protocol treatment is to begin within 28 days of enrollment.

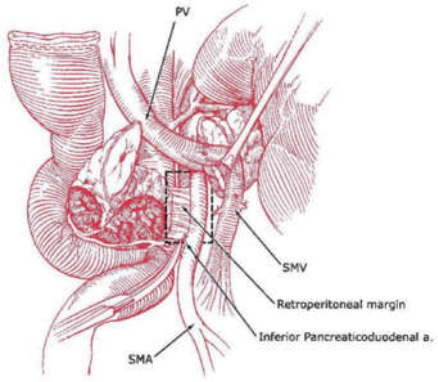
Treatment Schematic



- The first three patients will not receive Cy/GVAX. They will only receive SBRT/FOLFIRINOX. Vaccine/Cyclophosphamide treatments: (patients 4-18) Administer cyclophosphamide (Cy) at 200 mg/m^2 intravenously over 30 minutes the day before each vaccine. Each vaccination (GVAX) will consist of six total intradermal injections of vaccine. Two injections are administered each in the upper right and left thighs, and two injections in the upper non-dominant arm. Each injection will consist of approximately 8.3x10^7 cells in a 0.7 to 0.73 ml solution. Vaccine must be initiated within 6-10 weeks from surgery.
- Adjuvant SBRT will commence 13-17 days after the first vaccine is administered. Patients receive 5 days of SBRT (6.6 Gy daily) over a one to two week period to the tumor bed (as delineated by surgical clips placed by the surgeon).
- FOLFIRINOX should start at a minimum of 1 week after completion of SBRT. See diagram above. FOLFIRINOX is given for 24 weeks, this can be given locally. Patients will be evaluated after the first 2 cycles to determine if there have been any DLTs. Vaccine #2 is given 35 days (+/- 7 days) after completion of FOLFIRINOX. Cy/Vaccines #3-5 are given every 28 days +/- 3 days. If patients are without evidence of recurrence they can qualify for additional Cy/vaccine boosts where they will receive each Cy/vaccine every 6 months (every 12 months with Amendment #10) +/- 30 days until recurrence, toxicity, or death.

8.1 Surgery

Pancreaticoduodenectomy must be performed such that clips are placed at the time of surgery. The surgical procedure performed will result in either a R0 or R1 resection as determined by the operating surgeon. Information regarding any surgical therapy will be recorded including the operation performed, whether vascular resection and reconstruction was required, completeness of the resection (R0 vs. R1), duration of the operation, blood loss, the length of stay and the need for re-admission within 30 days of surgery. Central pathology review for all patients will be conducted by Dr. Ralph Hruban. Pancreaticoduodenectomy is the standard of care to which vaccine therapy is added. Titanium clips (minimum 1) will be placed to delineate the retroperitoneal/SMA margin at the time of surgery for SBRT treatment planning. See figure above (Retroperitoneal/SMA margin). In the event a clip cannot be placed at the SMA margin, a large clip and/or stent will be placed at the pancreaticojejenostomy (PJ) anastamosis as a surrogate of the tumor bed. In all cases the clinical target volume will be defined using the location of clips, the pathology and operative reports, and consultation with the surgical oncologist to ascertain the location of possible microscopic disease. If clips and/or stent cannot be identified to delineate the tumor bed or PJ, the patient will not be eligible for the study.



8.2 Cyclophosphamide and Vaccine Dose #1

For patients receiving Cy/GVAX, vaccine must be initiated within 6-10 weeks from surgery.

8.2.1 Cyclophosphamide (Pre- Vaccine #1)

- All laboratory tests will be drawn as per Table 1 (section 7).
- Administer cyclophosphamide at 200 mg/m^2 intravenously over 30 minutes Cyclophosphamide should be administered on a Monday, Tuesday, Wednesday or Thursday.
- Parameters for dosing cyclophosphamide: ANC ≥ 1000/mm^3 and platelets ≥ 100,000/mm^3. If parameters not met: delay cyclophosphamide/vaccine by 1 week.
- Vital signs including, ECOG PS, weight, blood pressure, heart rate, respiratory rate and temperature, are to be recorded.
- Blood samples for immune assays should also be drawn as described in 7.1.1.

8.2.2 Vaccination (GVAX) #1

- Each vaccine will be administered the day following cyclophosphamide.
- All patients will be seen in the Oncology outpatient center for vaccine administration and monitoring.
- Patients will be monitored for 30 minutes following vaccination for evidence of acute reaction to the injected vaccine cells. Vital signs: Blood pressure, heart rate, respiration rate and temperature will be recorded before each vaccination. BP, heart rate, respiration rate and temperature will be recorded again after each vaccine administration.

- Administration of vaccine: Each vaccination will consist of a total of 6 intradermal injections, two each in the upper right and left thighs, and two in the upper non-dominant arm. Each injection will consist of approximately $8.3x10^7$ cells in a 0.7 to 0.73 ml solution. The volume of the vaccination dose is approximate. A lidocaine based topical anesthetic (may include but is not limited to EMLA or ELA-MAX) cream will be applied to each injection site at least one hour prior to vaccination to diminish the discomfort associated with the intradermal injections. Omit lidocaine if allergic. See **Appendix I** for further details.

8.3 SBRT administration

- The first 3 patients will initiate SBRT 6-10 weeks post-surgery.
- The remaining 15 patients will begin SBRT between 13-17 days following Vaccine #1.
- SBRT will be initiated (Monday-Friday) and delivered for one week (preferred), although if necessary SBRT can be given over two weeks with at least 2 fractions per week.

  For schedule of activities and required laboratory tests, please see Table 2, section 7.

8.3.1 Radiation Treatment information:

Clip placement to outline pancreatic tumor bed:

Treatment on this protocol requires placement of titanium clips outlining the tumor bed (retroperitoneal/SMA margin) for targeting purposes (see diagram above). All surgeons listed as co-investigators on the trial must agree to place clips at the time of surgery, otherwise the patient will not be eligible. They will place 1 clip at the superior and inferior area of the SMA margin and one in the center of the superior/inferior clips.

8.3.2 Simulation:

1) Patients can undergo simulation >=3 weeks following surgical resection. Patients are placed supine in an Alpha Cradle or equivalent immobilization.
2) Standard free-breathing CT and/or respiratory-correlated 4-D pancreatic protocol CT will be obtained on each patient. The 4D-CT scan will be used for characterizing target motion during quiet respiration. For more accurate delineation of anatomy an arterial phase pancreatic protocol CT may be obtained (optional, typically during expiration breath hold, 1.25 mm slices). Clip to clip fusions between these scans should be utilized whenever possible. The simulation scan should include T4/T5 to L5/S1 (upper abdomen).
3) IV and oral contrast must be used for simulation, unless contraindicated.
4) Specialized compression belts (developed at MSKCC) may be utilized for some patients. They come in 4 sizes: S, M, L, and XL. Each belt has an adjustable pressure cuff which can be used to reduce breathing motion. Fluoroscopy is used to assess motion of surgical clips before and after compression. The goal is to reduce motion from typically 11 - 22 mm peak to less than 5 mm. If the fiducial motion cannot be decreased to 5 mm or less, then respiratory gating using either the Varian Respiratory Management (RPM) system or the Elekta Active Breathing Coordinator (ABC) will be utilized for treatment delivery. Prior to simulation, standard guidelines will be followed. Abdominal compression devices are also acceptable.

Patients may be treated either with respiratory gated or breath hold (ABC) (treatment using Elekta LINAC based treatment

8.3.3 Treatment planning:

1) An SBRT treatment plan will be developed using Eclipse™, Multi-Plan™, Pinnacle™ or equivalent treatment planning system based on tumor geometry and location at each institution. Institutional standards for radiation quality assurance and radiation delivery will be utilized.
2) Ideally, all patients will receive five fractions of 6.6 Gy delivered over a five day period. Ideally all 5 fractions should be delivered Monday thru Friday, however it may be delivered over two weeks as long as the patient receives at least two fractions a week.
3) The clinical treatment volume (CTV) will be based on the surgical clips and pre-surgical scan. The final CTV will be defined by the attending radiation oncologist after reviewing the diagnostic CT, respiratory-correlated 4D-CT scan, pancreas protocol CT, and the FDG-PET/CT scan (pre-surgical if available). The clips will be used to outline the CTV. These scans will be used to define the ITV (internal target volume). The final PTV (planning treatment volume) expansion will consist of an additional 1 cm of margin expansion, except if the margin results in expansion into the stomach. In these cases, margin expansion is allowed to be non-uniform. The dose will be prescribed to the isodose line that completely surrounds the PTV. It is recommended that 5-12 co-planer fields be used in the radiation treatment plan.
4) Contours of the clips used for target localization will be generated on the applicable image sets, to be used for patient setup on treatment (cone beam CT). Radiation dose to the adjacent normal tissue will be minimized.

   Normal tissues will be limited as follows:
   - Liver (excluding tumor): 50% should be limited to <12 Gy
   - Kidney: Combined volume for both should have 75% <12 Gy
   - Stomach: V15<9cc and V20<3cc. 50% should be limited to <12 Gy (no more than 1 cc of stomach can receive >33 Gy)
   - Spinal Cord max: no more than 1cc can receive >8 Gy

5) No more than 1cc of the PTV can receive >130% of the prescription dose (4920 cGy).
6) Greater than 90% of the PTV should receive 100% of the prescription dose (3300 cGy).
   If above constraints cannot be made then 100% of the GTV must receive at least 25 Gy (an allowed minor deviation and will be documented). If this constraint cannot be met, the patient should be removed from the protocol.

   Radiation dose to the adjacent normal tissue will be minimized.
   Based on an analysis of duodenal/bowel toxicity representing pooled data from 3 previous prospective studies, the following dose constraints must be met:
7) All patients must start treatment within 4 weeks of the simulation scan.

8.3.4 Treatment Delivery (LINAC based):

1) Initial patient positioning will be based on volumetric kV (cone-beam CT) imaging with shifts to bony anatomy as appropriate.
2) Orthogonal kV/MV or kV/kV projection imaging will be used to verify the location of the clips prior to delivery of first treatment beam. A secondary shift based on the location of clips may be utilized, as indicated by the position of the clips. For

free-breathing treatments, kV fluoroscopic images should be obtained to confirm the anticipated position of these clips during the entire respiratory cycle.

3) Active monitoring of treatment delivery accuracy will be accomplished using kV and/or MV projection imaging, either immediately before or during all (or a subset of) treatment fields.
4) Patient-specific dosimetric QA will be performed as per standard practice at each participating institution.

Following SBRT, all patients will be monitored clinically and with serial imaging (CT scans and PET/CT if possible and as needed). Please refer to **Tables 2** and **3** for a schedule of the medical history and physical examinations, complete blood count (CBC), comprehensive chemistry panel, tumor marker studies, and blood sample collection for research efforts to develop novel serum biomarkers.

8.4 FOLFIRINOX 24 weeks

8.4.1 FOLFIRINOX administration (After Vaccine #1)

- Patients will begin FOLFIRINOX 7-28 days post SBRT
- Patients in the second cohort (receiving Cy/GVAX) may opt to have cycles 4-6 of FOLFIRINOX locally, with the treating physician responsible for monitoring toxicities and determining any dose reductions. Efforts will be made to obtain CT scans, CA19-9 labs, and toxicities collected during this time. For AE reporting during local treatment, please see section 15.3.
- For schedule of activities and blood draws, please see **Table 3**. For parameters for FOLFIRINOX dosing, please see **Table 5**.

- **FOLFIRINOX Administration:**

Treatment will be administered on an outpatient basis. Reported adverse events and potential risks for FOLFIRINOX are described in **Section 9.5**. Appropriate dose modifications for FOLFIRINOX are described in **Section 9.4**. No investigational or commercial agents or therapies other than those described below may be administered with the intent to treat the patient's malignancy. Qualified personnel who are familiar with procedures that minimize undue exposure to them and to the environment should undertake the preparation, handling, and safe disposal of chemotherapeutic agents in a self-contained, protective environment. Discard unused portions of injectable chemotherapeutic agents supplied as single-dose preparations within eight hours of vial entry to minimize the risk of bacterial contamination. The total administered dose of chemotherapy may be rounded up or down within a range of 5% of the actual calculated dose.

Table 5: FOLFIRINOX REGIMEN DESCRIPTION					
Agent	**Premedications; Precautions**	**Dose**	**Route**	**Schedule** (-1/+3 days)	**Cycle Length**
Oxaliplatin	Avoid exposure to cold (food, liquids, air) for 24 hr after each dose.	85 mg/m^2 in 500 cc D5W	IV over approximately 2 hours	Days 1 and 15, every 28 days	One cycle is approx. 28 days
Irinotecan	Atropine should be immediately available for anti-cholinergic reactions	180 mg/m^2 in 500cc D5W	IV over approximately 90 minutes	Days 1 and 15, Every 28 days	
Leucovorin		400 mg/ m^2 in 250cc NS	IV over approximately 2 hours	Day 1and 15, Every 28 days	
5-FU infusion		2,400 mg/m^2	IV over approximately 46 hours	Days 1 and 15, Every 28 days	
Peg-Filgrastim	May experience bone pain	6 mg	SQ	24-72 hours after the end of each chemotherapy dose (Every 2 weeks or as clinically indicated)	

8.5 Cyclophosphamide/Vaccine #2-5 and Beyond (following completion of 24 weeks of FOLFIRINOX chemotherapy)

*****All but the first 3 patients will receive Cy/GVAX.**

8.5.1 Pre-Vaccine Cyclophosphamide

- Please see Table 4 for schedule of blood draws and other activities. CBC and CMP to be drawn within 1 week of cyclophosphamide dosing.
- Cyclophosphamide to be administered on a Monday, Tuesday, Wednesday or Thursday.
- Parameters for dosing cyclophosphamide: ANC ≥ 1000/mm^3 and platelets ≥ 100,000/mm^3. If parameters not met: delay cyclophosphamide/vaccine by 1 week.
- Administer cyclophosphamide at 200 mg/m^2 intravenously over 30 min.
- Vital signs including ECOG PS, weight and, blood pressure, heart rate, respiration rate, and temperature are to be recorded.
- Blood samples for immune assays should also be drawn as described in 7.1.1.

8.5.2 Vaccine (GVAX) #2-5

- Vaccines #3-5 will be administered at 28 day intervals (+/- 3 days) following vaccine #2. For vaccine #2, this time frame is also gauged as being approximately

35 days (+/- 7 days) following completion of the final dose of adjuvant FOLFIRINOX.

- Please see Table 4, section 7 for schedule of laboratory and other tests.

8.5.3 Vaccine (GVAX) #6 and thereafter until recurrence

- If patient has no evidence of disease, they can qualify for additional cy/vaccine boosts (Vaccine #6 +) where Cy/vaccine are delivered every 6 months (+/- 30 days). As of Amendment #10, boost vaccinations will be administered annually (+/- 30 days). Up to 28 days prior to every vaccination, all patients will undergo a re-staging scan, CT chest/abdomen/pelvis (or MRI abdomen/pelvis with contrast and non-contrast chest CT) and CA 19-9, complete blood count and differential, comprehensive panel.

- Please see Table 4, section 7 for schedule of laboratory and other tests.

- Blood samples for immune assays should also be drawn as described in 7.1.1. As of Amendment #9, research bloods are no longer required to be collected.

8.6 Off Study and Follow-up

8.6.1 Criteria for Removal from Study

Patients will be taken off study if one of the following criteria applies:

- Recurrent or metastatic disease
- Patient chooses not to receive additional vaccine boosts (Vaccine #6+) or elects to withdraw from the study
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse events
- Changes in the subject's condition render the subject unacceptable for further treatment in the judgment of the investigator

8.6.2 Off Study Visit

The following evaluations are recommended (but not required) at the off study visit:

- History and Physical exam with vitals and ECOG performance status
- Assessment of toxicities (including vaccine site reactions)
- Heme-8 with differential, including platelets
- Comprehensive panel including electrolytes, BUN, creatinine, AST, ALT, alkaline phosphatase, and total bilirubin.
- CA19-9
- Correlative tests that include up to 180 cc of blood for immune monitoring and up to 20 cc of blood for serum banking. As of Amendment #9, research bloods are no longer required to be collected.
- CT scan abdomen/pelvis and chest. If done within 30 days the CT scans do not need to be repeated. (If allergic to CT scan contrast, obtain abdominal/pelvic MRI with contrast and non-contrast chest CT)

8.6.3 Follow-Up

If consent is granted, patients who received the pancreatic tumor vaccine will be followed annually via a separate protocol entitled, "Long-term follow-up of patients who receive

lethally irradiated allogeneic pancreatic tumor cells transfected with the GM-CSF gene." Consent can be obtained at any point in the protocol. It is recommended that patients schedule follow-up visits at the discretion of the patient's local oncologist and the results sent to us if the patient agrees. All attempts will be made to obtain disease-free and overall survival data on each patient, including those not receiving vaccine.

8.7 General Concomitant Medication and Supportive Care Guidelines

8.7.1 Radiation Therapy (SBRT)

- Use of acid-blocking agents such as proton pump inhibitors is required during SBRT and is recommended to continue for 6 months following SBRT.

- Anti-emetic pre-medication, if needed, should be administered per institutional guidelines.

- Use of high dose antioxidants is not permitted during radiation therapy.

- Use of moisturizing creams and other topically applied medications and ointments should not be used within the radiation ports without prior approval from the radiation oncologist.

8.7.1.1 Anti-diarrheals and Anti-emetics

For symptoms of diarrhea and/or abdominal cramping that occur within 2 weeks after SBRT, patients will be instructed to begin taking loperamide. Additional anti-diarrheal measures may be used at the discretion of the treating physician. Patients should be instructed to increase fluid intake to help maintain fluid and electrolyte balance during episodes of diarrhea.

For symptoms of nausea and vomiting, it is recommended that patients take Compazine or Zofran one hour prior to SBRT and on a PRN basis. These medications will continue for 5 days following SBRT. Patients will be instructed to increase fluid intake.

8.7.1.2 Other Concomitant Medications

Therapies considered necessary for the patient's well-being may be given at the discretion of the investigator. Other concomitant medications should be avoided except for analgesics, chronic treatments for concomitant medical conditions, or agents required for life-threatening medical problems. In general, these medications will be prescribed by the attending medical oncologist.

8.7.1.3 Supportive Care Guidelines

In case participants develop nausea/vomiting/diarrhea or myelosuppression, supportive medications will be prescribed as per Clinical Center and ASCO guidelines. Seizures were seen in some animal toxicology studies, although at doses much higher than those anticipated for this study. Seizures in animals were successfully treated with lorazepam.

8.7.1.3.1 Risk Information

It is difficult at this time to predict with confidence the percentage rate of complications from the proposed SBRT treatment. However, it is reasonable to extrapolate from the current experience with radiotherapy in and around the pancreas. Based upon prior phase I and phase II studies, we anticipate that the toxicities associated with this treatment will be acceptable.

Toxicity commonly associated with such treatment includes nausea, vomiting, anorexia and weight loss. Severe side effects such as gastrointestinal (GI) obstruction,

perforation, or hemorrhage are uncommon complications, occurring in <5% of patients undergoing standard radiation therapy for pancreatic cancer. Although we expect a comparable rate of complications with fractionated radiotherapy, it is important to note that vomiting, GI obstruction, GI hemorrhage, anorexia and weight loss are also commonly associated with pancreatic cancer progression. Clinical and radiographic assessments will be performed in an effort to identify these effects, ascertain their etiology and provide the most appropriate palliative measures. Hepatic and renal toxicity is not anticipated given the expectation of limited incidental irradiation of these organs. Complications, if any, will be graded according to the CTCAE, National Cancer Institute, version 4.0.

8.7.2 Chemotherapy (FOLFIRINOX)

8.7.2.1 Anti-emetics

Patients should be pre-medicated prior to the FOLFIRINOX treatment with anti-emetics per institutional guidelines.

Agents that may be considered include, but are not limited to: promethazine, prochlorperazine, metoclopramide, haloperidol, droperidol, lorazepam and serotonin-antagonists (e.g. ondansetron, granisetron, dolasetron).

Delayed emesis, should it occur, will be treated for future FOLFIRINOX chemotherapy doses with a delayed emesis regimen (e.g. 3 days of metoclopramide + dexamethasone, 3 days of serotonin antagonist, or use of long-acting serotonin antagonist).

Due to the emetogenic nature of this regimen, aprepiant will be administered with each cycle in addition to concomitant anti-emetics at the discretion of the treating physician.

8.7.2.2 Anti-diarrheal

Loperamide: For symptoms of diarrhea and/or abdominal cramping that occur at any time during a treatment cycle patients will be instructed to begin taking loperamide. Loperamide should be started at the earliest sign of (1) a poorly formed or loose stool or (2) the occurrence of 1 to 2 more bowel movements than usual in 1 day or (3) an increase in stool volume or liquidity. Loperamide should be taken in the following manner: 4 mg at the first onset of diarrhea, then 2 mg every 2 hours around the clock until diarrhea-free for at least 12 hours. Patients may take loperamide 4 mg every 4 hours during the night. The maximum daily dose of loperamide is 16 mg/day.

Antibiotics: Oral fluoroquinolone treatment may be initiated for ANC<500 or diarrhea for >24 hours despite loperamide, at the discretion of the treating physician.

8.7.2.3 Anticoagulants

Prophylactic or therapeutic doses of Coumadin or low-molecular weight heparin are permitted. Low-dose aspirin (≤ 325 mg/d): Aspirin may be continued if the patient was on this prior to enrollment.

8.7.2.4 Growth Factors

Peg-filgrastim (Neulasta) will be administered 24-72 hours after the end of each chemotherapy dose, approximately every 2 weeks (2x per cycle) or as clinically indicated at the discretion of the treating physician.

The use of erytropoetin EPO in this protocol is permitted at the discretion of the treating physician. However, these agents are not recommended.

8.7.2.5 Neurotoxicity Management

Ca++/Mg++: Various agents have been used in an attempt to minimize neurotoxicity of oxaliplatin (e.g. Mg++, Ca++). Their routine use requires further confirmation of efficacy. They may be used in this study at the discretion of the treating physician.

8.7.2.6 Supportive Care Guidelines

All supportive measures consistent with optimal patient care will be given throughout the study.

9.0 Dose Modifications And Management of Toxicity

All toxicities should be graded according to the Common Terminology Criteria for Adverse Events (CTCAE) (version 4).

9.1 Cyclophosphamide Dose Modifications

No dose modifications will be made for cyclophosphamide. The cyclophosphamide dose is fixed at 200 mg/m^2 IV, delivered over 30 minutes. Parameters for treatment within 1 week of planned dose (or 28 days for cy/vaccine doses 6 and beyond): ANC ≥ 1000/mm^3 and platelets ≥ 100,000/mm^3.

9.2 Vaccine Dose Modifications

No dose modifications will be made for the vaccine.

9.3 Dose Modifications for Obese Patients

There is no clearly documented adverse impact of treatment of obese patients when dosing is performed according to actual body weight. Therefore, all chemotherapy dosing (except cytoxan) is to be determined solely by actual weight without any modification unless explicitly described in the protocol. This will eliminate the risk of calculation error and the possible introduction of variability in dose administration. Failure to use actual body weight in the calculation of drug dosages will be considered a major protocol deviation. Physicians who are uncomfortable with calculating doses based on actual body weight should recognize that doing otherwise would be a protocol violation. **Please note: All chemotherapy agents will be dosed per BSA.**

9.4 FOLFIRINOX Dose Modifications

Adapted from Conroy, T et al. FOLFIRINOX versus Gemcitabine for Metastatic Pancreatic Cancer. N Engl J Med 2011; 364:1817-1825.

Dosage adjustment guidelines for toxicities with FOLFIRINOX
If more than one of the dose modifications apply, use the most stringent (i.e., the greatest dose reduction). Dose modifications are cumulative and permanent. Note that the dose of Leucovorin does not change with FOLFIRINOX dose level reductions, but is dependent on pharmacy supply.

If treatment is held for any of the below toxicities for ≥ 3 weeks, patients should discontinue all protocol therapy. Patients are off study if they develop the same grade 4 toxicity despite a first dose reduction.

Dose levels for FOLFIRINOX

Dose Level	**Oxaliplatin** (mg/m^2)	**Irinotecan** (mg/m^2)	**Leucovorin**[1] (mg/m^2)	**5-FU Infusion** (mg/m^2)
0	85	180	400	2400
-1	65	150	400	1920
-2	50	120	400	1600
-3	40	100	400	1360

[1]Dose dependent upon pharmacy supply

9.4.1 Hematologic toxicity

Do not dose reduce until the granulocyte count is ≤ 1.0 x 10^9/L and the platelet count is ≤75 x 10^9/L. Note: FOLFIRINOX dose modifications for hematologic toxicity are not based on CTCAE severity grades.

For ANC < 1,000/mm^3: Delay FOLFIRINOX until ANC ≥ 1,000/mm^3, then resume FOLFIRINOX with one dose level reduction for all subsequent cycles.

For Febrile Neutropenia (defined as ANC < 1,000/mm^3 and temperature ⩾ 100.5°F): Delay FOLFIRINOX until resolution of fever and ANC ≥ 1,000/mm^3, then resume FOLFIRINOX with one dose level reduction for all subsequent cycles.

For Platelets 50,000 – 75,000/mm^3: Delay FOLFIRINOX until platelets ≥ 75,000/mm^3, then resume FOLFIRINOX at the same dose level.

Second or More Occurrence of platelets 50,000 - 75,000/mm^3: Delay FOLFIRINOX until platelets ≥ 75,000/mm^3, then resume FOLFIRINOX with one dose level reduction for all subsequent cycles.

For Platelets < 50,000/mm^3: Delay FOLFIRINOX until recovery to Plts ≥ 75,000/mm^3, then resume FOLFIRINOX with one dose level reduction for all subsequent cycles.

9.4.2 Gastrointestinal toxicities

Patients must be instructed in the use of loperamide as treatment for diarrhea, and must have a supply of this drug upon starting FOLFIRINOX. Patients should not be retreated with irinotecan until diarrhea has resolved to ≤ grade 2 with maximal medical management within 5 days. Patients should be optimally managed with anti-diarrheal or anti-emetic medications before dose modifications are made.

For Grade 2 Diarrhea (despite optimal medical management):

First Occurrence: Delay FOLFIRINOX until recovery to grade ⩽ 1 or baseline, then resume FOLFIRINOX at the same dose level.

Second or More Occurrence: Delay FOLFIRINOX until recovery to grade ⩽ 1 or baseline, then resume FOLFIRINOX with one dose level reduction for all subsequent cycles.

For Grade 3 Diarrhea (despite optimal medical management):

First Occurrence: Delay FOLFIRINOX until recovery to grade ⩽ 1 or baseline, then resume 5-FU and oxaliplatin at the same dose level and irinotecan with one dose level reduction for all subsequent cycles.

Second or More Occurrence: Delay FOLFIRINOX until recovery to grade ⩽ 1 or baseline, then resume FOLFIRINOX with one dose level reduction for all subsequent cycles.

For Grade 4 diarrhea (despite optimal medical management):
Delay FOLFIRINOX until recovery to grade ⩽ 1 or baseline then resume FOLFIRINOX with one dose level reduction for all subsequent cycles.

Nausea/Vomiting The following dose modifications are based on toxicity experienced during a cycle.

For Grade 3 Nausea/Vomiting (despite optimal medical management):

First Occurrence: Delay FOLFIRINOX until recovery to grade ⩽ 1 or baseline, then resume 5-FU at the same dose level and oxaliplatin and irinotecan with one dose level reduction for all subsequent cycles.

Second or More Occurrence: Delay FOLFIRINOX until recovery to grade ⩽ 1 or baseline, then resume FOLFIRINOX with one dose level reduction for all subsequent cycles.

For Grade 4 Nausea/Vomiting (despite optimal medical management):
Delay FOLFIRINOX until recovery to grade ⩽ 1 or baseline, then resume FOLFIRINOX with one dose level reduction for all subsequent cycles.

Mucositis The following dose modifications are based on toxicity experienced at any time during a cycle.

Grade 3 Mucositis:

First Occurrence: Delay FOLFIRINOX until recovery to grade ⩽ 1, then resume irinotecan and oxaliplatin at the same dose level and 5-FU with one dose level reduction for all subsequent cycles.

For Second or More Occurrence: Delay FOLFIRINOX until recovery to grade ⩽ 1, then resume FOLFIRINOX with one dose level reduction for all subsequent cycles.

Grade 4 Mucositis: Delay FOLFIRINOX until recovery to grade ⩽ 1, then resume FOLFIRINOX with one dose level reduction for all subsequent cycles.

9.4.3 Peripheral Sensory Neuropathy

For paresthesia/dysethesia interfering with function and persisting between treatments: Decrease oxaliplatin by one dose level for all subsequent cycles.

For painful paresthesia/dysesthesia or symptoms that interfere with function and ADL, but improve (no longer painful or no longer interfering with ADL) between treatments: Decrease oxaliplatin by one dose level for all subsequent cycles.

For painful paresthesia/dysesthesia or symptoms that interfere with function and ADL that persists between treatments: Discontinue oxaliplatin.

For persistent disabling or life-threatening paresthesia/dysesthesia: Discontinue oxaliplatin.

For pharyngo-laryngeal dysesthesia: Increase the duration of oxaliplatin infusion to 3 hours for subsequent cycles**.**

9.4.4 Venous Thromboembolic Events

For Grade 2 or 3 venous thromboembolic event: Continue FOLFIRINOX at the same dose level. Do not use warfarin for therapeutic anticoagulation; use of low molecular weight heparin is allowed.

Grade 4 venous thromboembolic event: Discontinue FOLFIRINOX.

9.4.5 Cardiac toxicity

In case of angina pectoris or of myocardial infarction, 5FU has to be stopped.

9.4.6 Increase of bilirubin

In case of elevation of bilirubin, it is suggested to exclude an obstruction of the biliary stent or progressive disease and to postpone chemotherapy.

For Grade 2 Increased Bilirubin: Skip irinotecan until bilirubin improves to $\leqslant$ grade 1. For hyperbilirubinemia considered at least possibly related to irinotecan, resume irinotecan with one dose level reduction for all subsequent cycles.

For hyperbilirubinemia considered unrelated to irinotecan, resume irinotecan at the previous dose level.

For Grade 3 or 4 Increased Blood Bilirubin: Delay FOLFIRINOX until bilirubin improves to $\leqslant$ grade 1. If bilirubin is thought to be due to a chemotherapy drug, then resume that drug at the next lower dose level and the other drugs at the same dose level when total bilirubin improves to $\leqslant$ grade 1.

For hyperbilirubinemia considered unrelated to treatment (all drugs), resume FOLFIRINOX at the previous dose levels.

9.4.7 Allergic Reactions

For grade 2 allergic reactions: Interrupt infusion(s). Manage reaction according to institutional policy. Restart the infusion(s) when symptoms resolve to $\leqslant$ grade 1 and pretreat before all subsequent doses.

For grade 3 or grade 4 allergic reactions: Discontinue infusion. Manage reaction according to institutional policy. Discontinue FOLFIRINOX.

9.4.8 Other toxicities

Any other toxicity $\geq$ grade 2, except anemia and alopecia, can justify a reduction of dose if medically indicated.

For all other grade 3 non-hematologic toxicities considered at least possibly related to FOLFIRINOX: Skip the responsible drug(s) until toxicity improves to ≤ grade 1, then resume the responsible drug(s) with one dose level reduction for all subsequent cycles.

For grade 4 non-hematologic toxicities considered at least possibly related to FOLFIRINOX: Discontinue the responsible drug(s).

9.5 FOLFIRINOX Toxicities

9.5.1 Oxaliplatin [Eloxatin] Toxicity

The most commonly observed oxaliplatin toxicities include neurotoxicity, GI toxicity, and myelosuppression. Three neurotoxicity syndromes have been seen:

- Acute sensory neuropathy develops within hours to 2 days after oxaliplatin administration. Symptoms include paresthesias, dysesthesias, and hypothesia of the hands, feet and perioral regions. Jaw spasm, abnormal tongue sensation, dyarthria, eye pain and a sensation of chest pressure have also been noted. Acute sensory neuropathy symptoms may be exacerbated by exposure to cold temperature or cold objects. Symptoms are reversible, usually resolving within 14 days and commonly recurring with further dosing. This syndrome has been observed in about 56% of patients receiving oxaliplatin with 5-FU and leucovorin.

- Acute pharyngolaryngeal dysesthesia is reported to occur in 1-2% of patients. This syndrome is characterized by a subjective sensation of difficulty breathing or swallowing without laryngospasm or bronchospasm or objective evidence of hypoxia. Avoidance of cold drinks, food and air is suggested in order to minimize pharyngolaryngeal dysesthesia. Antianxiety agents (e.g. lorazepam) may be used to treat pharyngolaryngeal dysesthesias once oxygen saturation has been documented to be normal.

- Peripheral neuropathy persisting > 14 days is characterized by paresthesias, dysesthesias, and hypothesia. Abnormalities in proprioception may also be seen. Symptoms of persistent neuropathy may improve upon discontinuation of oxaliplatin.

- Various agents have been used in an attempt to minimize neurotoxicity of oxaliplatin (e.g. carbamazepine, Mg++, Ca++). Their routine use requires further confirmation of efficacy. They may be used in this study at the discretion of the treating physician.

- Gastrointestinal toxicities include nausea, vomiting (oxaliplatin is considered to be moderately emetogenic) and diarrhea.

- Neutropenia is reported in 73% of patients receiving oxaliplatin with 5-FU and leucovorin (44% grade 3 or 4). Grade 3 or 4 thrombocytopenia is reported to occur in 4% of patients receiving the combination.

- Allergic reactions, similar to those seen with other platinum compounds, have also been observed in patients treated with oxaliplatin. Reactions range from rash to anaphylaxis

- Rarely, oxaliplatin has been associated with pulmonary fibrosis, which may be fatal. Oxaliplatin should be discontinued in the presence of unexplained pulmonary symptoms (e.g. nonproductive cough, dyspnea) or pulmonary

infiltrates until interstitial lung disease or pulmonary fibrosis have been ruled out.

- Recent reports of oxaliplatin extravasation suggest that tissue necrosis may result and that oxaliplatin should be considered a vesicant. No standard treatment exists for oxaliplatin extravasation although heat and sodium thiosulfate have both been suggested.

- Veno-occlusive disease (VOD) of the liver is a rare complication associated with oxaliplatin and 5-FU. Clinical manifestations of VOD include hepatomegaly, ascites, and jaundice. Histologically, VOD is characterized by diffuse damage in the centrilobular zone of the liver. Sequelae of VOD include hepatomegaly, splenomegaly, portal hypertension, and esophageal varices. A recent analysis of resected liver metastases in 153 patients indicated histological findings consistent with VOD in 6/27 patients who received 5-FU alone, 4/17 patients who received 5-FU and irinotecan, 20/27 patients who received 5-FU and oxaliplatin, and 14/16 who received 5-FU, oxaliplatin and irinotecan. The remaining 66 patients had not received chemotherapy prior to resection. There were no such findings in these patients.

For more information on toxicities associated with oxaliplatin, please see the package insert (**Appendix III)**.

9.5.2 5-Fluorouracil (5-FU: fluorouracil: Adrucil®) Toxicity

- Nausea, diarrhea, vomiting (mild);
- Stomatitis (5-8 days after treatment initiation);
- Myelosuppression; granulocytopenia (9-14 days); thrombocytopenia (7-14 days);
- Alopecia; loss of nails; hyperpigmentation;
- Photosensitivity; maculopapular rash; palmar-plantar erythrodysethesias: (42-82% receiving continuous infusion);
- CNS effects: cerebral ataxia (rare);
- Cardiotoxicity: MI, angina: asymptomatic S-T changes 68%;
- Ocular effects: excessive lacrimation and less commonly, tear duct stenosis.

Drug Interactions
Leucovorin enhances the cytotoxicity of 5-FU by forming a more stable tertiary complex with thymidylate synthase. Concomitant administration of 5-FU with warfarin has been reported to result in increased INR/prolonged prothrombin time. Patients receiving both drugs should be followed with weekly INRs.

Please refer to the package insert for complete product information (**Appendix IV)**.

9.5.3 Leucovorin Calcium (Folinic Acid; calcium folinate; citrovorum factor; N 5-formyltetrahydrofolate; 5-formyl-FH4; folinic acid) Toxicity

The only adverse reactions associated with leucovorin are allergic reactions. These are extremely uncommon.

Please refer to the package insert for complete product information (**Appendix V)**.

9.5.4 Irinotecan (CPT-11, Camptosar) Toxicity

Virtually all phase I and II studies of irinotecan have reported neutropenia and/or late diarrhea (diarrhea occurring more than 24 hours after the irinotecan administration as the dose limiting toxicities (depending on the schedule).

- Bone marrow suppression: [U.S. Boxed Warning]: May cause severe myelosuppression. Deaths due to sepsis following severe myelosuppression have been reported. Routine administration of a colony-stimulating factor is generally not necessary, but may be considered for patients experiencing significant neutropenia.
- Colitis: Colitis, complicated by ulceration, bleeding, ileus, and infection has been reported.
- Diarrhea: [U.S. Boxed Warning]: Severe diarrhea may be dose-limiting and potentially fatal; two severe (life-threatening) forms of diarrhea may occur. Early diarrhea occurs during or within 24 hours of receiving irinotecan and is characterized by cholinergic symptoms (e.g., increased salivation, diaphoresis, abdominal cramping); it is usually responsive to atropine. Late diarrhea occurs more than 24 hours after treatment which may lead to dehydration, electrolyte imbalance, or sepsis; it should be promptly treated with loperamide. Patients with diarrhea should be carefully monitored and treated promptly.
- Hypersensitivity reactions: Severe hypersensitivity reactions have occurred.
- Renal toxicity: Renal impairment and acute renal failure have been reported, possibly due to dehydration secondary to diarrhea.

Disease-related concerns:

- Bowel obstruction: Patients with bowel obstruction should not be treated with irinotecan until resolution of obstruction.
- Hepatic impairment: Use with caution in patients with hepatic impairment.
- Hyperbilirubinemia: Patients with even modest elevations in total serum bilirubin levels (1-2 mg/dL) have a significantly greater likelihood of experiencing first-course grade 3 or 4 neutropenia than those with bilirubin levels that were <1 mg/dL. Patients with abnormal glucuronidation of bilirubin, such as those with Gilbert's syndrome, may also be at greater risk of myelosuppression when receiving therapy with irinotecan. Use caution when treating patients with known hepatic dysfunction or hyperbilirubinemia; dosage adjustments should be considered.

Other less severe toxicities include:

- Nausea and vomiting;
- Anorexia, abdominal cramping;
- Alopecia, asthenia;
- Lymphocytopenia, anemia;
- Dehydration has occurred as a consequence of diarrhea, particularly when associated with severe vomiting.
- Cholinergic reactions: Patients may have an acute syndrome of lacrimation, diaphoresis, abdominal cramping, and diarrhea (early diarrhea) during or shortly after irinotecan administration; this syndrome is thought to be cholinergically mediated, and may be treated and subsequently prevented with atropine.
- Pulmonary toxicity, manifested as shortness of breath, non-productive cough and transient infiltrates on chest x-ray have been reported.
- Mucositis or colitis (sometimes with gastrointestinal bleeding);
- Occasionally, abnormalities of serum creatinine, hepatic enzymes, or thrombocytopenia have been observed.

Please refer to the package insert for complete product information (**Appendix VI)**.

10.0 Companion Studies

There will be one correlative substudy and all patients are encouraged to participate.

10.1 Correlative Science

As described in 1.1, the John's Hopkin's group has completed three single institution clinical whole cell vaccine studies. Initially, the vaccine was tested in the adjuvant setting in a 14 patient phase I dose-escalation study(13). Results from that study showed that 3 of 8 subjects receiving the 2 highest doses of vaccine developed increased post-vaccination DTH-responses to autologous tumor. A functional genomic approach was developed that employed banked lymphocytes isolated from treated patients to screen differentially expressed pancreatic adenocarcinoma (PDA) antigens. This approach identified mesothelin as a novel PDA-associated T cell antigen. Using public domain computer algorithms, two mesothelin-derived epitopes each were identified that bound HLA-A0101, A0201, A0301 and A2402 (these four alleles covered all treated patients). Enhanced post-vaccination $CD8^+$ T cell responses to these epitopes were exclusively detected in the 3 patients demonstrating positive DTH responses. Importantly, these were the only patients on that study who demonstrated prolonged disease-free survival (>12 years). Thus, the induction of mesothelin-specific immunity appeared to be associated with disease-free survival (DFS)(13).

More recently, the whole cell vaccine was tested in a follow-up 60 patient adjuvant study testing the highest dose found to be bioactive in the phase I trial(12). Similar to the phase I study design(13), patients on this phase II study received an initial vaccination 8 weeks after pancreaticoduodenectomy, followed by chemoradiation, and then up to 4 more immunizations with the vaccine. Peripheral blood lymphocytes (PBL) from two patients demonstrating prolonged DFS were used to identify additional mesothelin epitopes by screening 15mer peptides overlapping by 10 amino acids covering the entire mesothelin protein sequence. The PBL used for epitope discovery were isolated from an HLA-$A0201^+$ and HLA-$A0101^+$ patient at 10 and 22.5 months following treatment completion respectively. Both patients were disease free for more than 2 years at the time of blood draw. Peptide library screening resulted in the identification of 4 new HLA-A0201-binding and 6 new HLA-A0101-binding mesothelin epitopes. $CD8^+$ T cell responses to the expanded set of mesothelin peptides were evaluated in 43 of the 60 treated patients whom expressed HLA-A1 and/or HLA-A2 (12). IFN-alpha ELISPOT analysis demonstrated that enhanced mesothelin-specific T cell responses were not observed following the 1st vaccination in patients with rapidly recurring disease. In addition, in patients remaining disease-free, the maintenance of enhanced mesothelin-specific responses throughout the course of treatment correlated with longer DFS. Furthermore, an expansion in the mesothelin-specific T cell repertoire following boosting vaccinations was observed almost exclusively in patients who demonstrated longer DFS. These data suggest that the induction, maintenance and expansion of the mesothelin-specific T cell repertoire may distinguish clinical responders from non-responders.

In the 3rd clinical study, the whole cell vaccine was tested in 50 patients with stage IV pancreas ductal adenocarcinoma refractory to Gemcitibine)(11). Patients enrolled in this study were divided into 2 cohorts. All patients received up to 6 whole cell vaccine treatments given every 3 weeks. However, 30 patients (cohort A) received vaccine alone and 20 patients (cohort B) received Cyclophosphamide (Cy) one day prior to each vaccination. Cy was combined with the vaccine because preclinical studies had shown that when given 1 day prior to vaccination, low doses of Cy can enhance vaccine-mediated immune induction(30)(30). This effect was mediated at least in part through the depletion of $CD4^+/CD25^+$ regulatory T cells (Tregs). Median survival in Cohort A and Cohort B were 2.3 months and 4.7 months respectively comparing favorably with what is reported for first and second line therapy in this patient population. IFNγ ELISPOT assays were used to measure $CD8^+$ T cell responses in the treated HLA-$A1^+$, HLA-$A2^+$ and HLA-$A3^+$ patients. These studies

demonstrated a trend toward prolonged progression-free survival in those patients who demonstrated persistent mesothelin-specific T cell response. Furthermore, HLA tetramer dilution analyses performed on a subset of treated HLA-$A2^+$ patients demonstrated that post-immunotherapy increases in the avidity of mesothelin-specific T cells were associated with prolonged survival(11).

More recently, the avidity of mesothelin-specific T cell responses in patients treated on the previously described 60 patient phase II adjuvant study have been assessed. Dilutional tetramer analysis was performed on PBL isolated prior to the first vaccination and following the first and final vaccinations from HLA-$A2^+$ subjects to assess the avidity of T cells specific for each of the 6 HLA-A0201-binding mesothelin epitopes. T cell avidity for each mesothelin peptide was plotted against DFS. **Figure 1** shows the relationship between the avidity of $MesoA2_{(530-538)}$-specific T cells and DFS. For each of the 6 mesothelin peptides evaluated, a trend toward improved DFS was observed in patients demonstrating higher avidity mesothelin-specific T cells. In addition, this trend was observed at each time point. However, although higher avidity mesothelin-specific T cells were sometimes present in pre-vaccination PBL, mesothelin-specific IFNγ responses were frequently not detected until after the first vaccination. Furthermore, in some patients, multiple boosts were required before both high avidity T cells and IFNγ responses could be detected. These data suggest that higher avidity pre-committed T cells are sometimes present pre-treatment in resected PDA patients and can be activated with vaccination. These data also suggest that these patients have up to a 10 fold increase in avidity compared to patients with metastatic disease that correlates with avidities measured in mice that clear tumors following vaccination with a GM-CSF-secreting vaccine. In addition, the distribution of avidities measured for each peptide was used to define cutoffs for distinguishing high from low avidity mesothelin-specific T cells. For this preliminary analysis, DFS>20 months vs. DFS<20 months was chosen as a binary clinical outcome parameter. **Figure 2** shows the number of mesothelin peptides for which high avidity T cells were detected for each patient evaluated. Interestingly, a larger repertoire of high avidity mesothelin-specific T cells was associated with improved DFS.

Collectively, the results from these 3 clinical studies have identified mesothelin as a relevant PDA T cell antigen and suggest that the induction and maintenance of mesothelin-specific immune responses is associated with prolonged survival. Furthermore, these studies suggest that two new T cell parameters, avidity and the expansion of the mesothelin-specific T cell repertoire, are key parameters for predicting prolonged disease-free survival. This new study will further assess the value of these T cell parameters in predicting DFS in patients at multiple institutions.

Figure 1. Meso $A2_{(530-538)}$-specific T cell Avidity vs DFS.

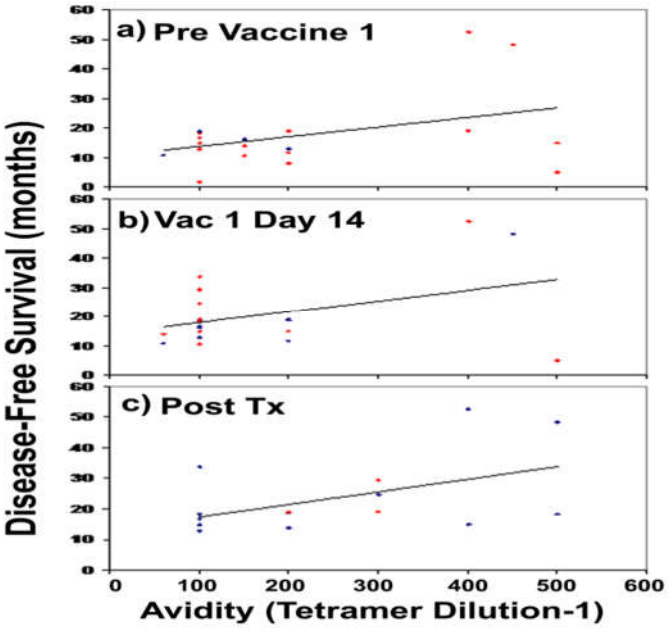


Figure 1. Dilutional MesoA2$_{(530-538)}$ tetramer analysis was performed on PBL isolated a) prior to vaccination, b) 14 days following the 1st vaccination or c) 28 days following the final vaccination from HLA-A2$^+$ patients. MesoA2$_{(530-538)}$-specific T cell avidity measured for each patient is shown plotted against DFS. Linear regressions were performed and the trend lines are shown. Blue data points were used when MesoA2$_{(530-538)}$-specific IFNγ responses were also detected. Red data points were used when IFNγ responses were not detected. Dilutions were performed using 1 μM tetramer stock solutions. Data is an example shown for all HLA-A2 patients for one HLA-A2 epitope. Summarized data for all HLA-A2 patients and for all 6 HLA-A2 epitopes is shown in Figure 2.

Figure 2. Longer Disease-Free Survival is Associated with Larger High Avidity Mesothelin-Specific CD8$^+$ T Cell Repertoires.

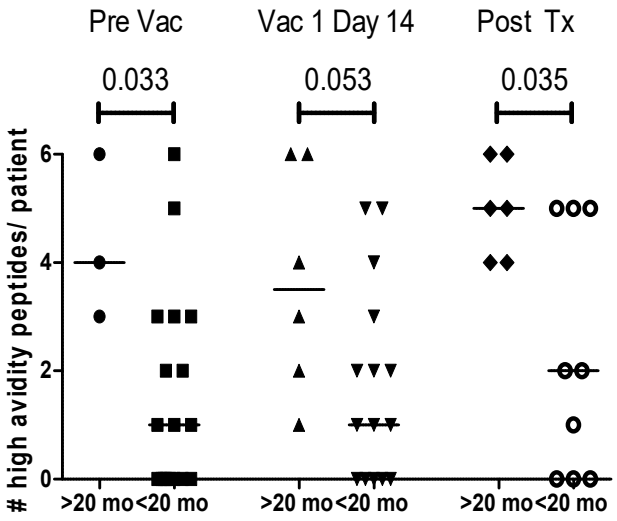


Figure 2. Dilutional tetramer analysis was performed for each of the 6 HLA-A2-binding mesothelin epitopes on PBL isolated prior to vaccination (Pre Vac), 14 days following the 1st vaccination (Vac 1 Day 14) or 28 days following the final vaccination (Post Tx) from HLA-A2$^+$ patients. Dilutions were performed using 1 μM tetramer stock solutions. Patients were divided into 2 groups: those who recurred within 20 months (< 20 mo) and those who remained disease-free for greater than 20 months (> 20 mo). Avidity dilution profiles were used to define cutoffs between high and low avidity T cells for each peptide. Shown are the numbers of peptides for which high avidity T cells were detected for each patient evaluated. High avidity repertoires were compared between groups using two-tailed Wilcoxon sign-rank tests and the calculated p-values are shown.

10.1.1 Objectives

Two key objectives for the correlative study are:

- To estimate the association of immune correlates to time to disease progression in patients with resected pancreatic cancer who receive the whole cell vaccine as part of their adjuvant therapy. The specific immune parameters include vaccine-induced changes in the number, function, avidity, size and diversity of the mesothelin-specific T cell repertoire.

10.1.2 Methods

The time points for collection of research bloods and the processing of samples are identified in the study calendar and described in detail in 7.0

Mesothelin-specific T cell analyses. We will assess mesothelin-specific T cell responses using an ELISPOT assay and dilutional HLA tetramer staining and flow cytometry analysis that are well established and have been successfully used to analyze immune responses in the prior phase I and II studies. Immune parameters to be studied include: the induction and maintenance of mesothelin-specific, cytokine secreting $CD8^+$ T cell responses, the evaluation of the size and diversity of the mesothelin-specific $CD8^+$ T cell repertoire against an expanded set of mesothelin T cell epitopes, and the assessment of the changes in avidity of the T cell repertoire with repeated vaccinations.

PBL. Lymphocytes will be obtained from peripheral blood before each immunotherapy treatment. Pre and post-treatment PBL will be isolated by Ficoll-Hypaque density gradient centrifugation. Isolated PBL will be stored frozen at -135°C in 90% AIM-V serum-free media containing 10% DMSO until the day of analysis. All specimens will be banked with a unique study identification number without any patient identifiers.

ELISPOT assay. HLA typing of all participants is performed at the Johns Hopkins Immunodiagnostics CORE facility. Mesothelin epitopes have so far been identified for HLA-A1, A2, A3 and A24. These four HLA alleles covered 82.3 percent of patients treated in the previously mentioned vaccine studies. However, it may be necessary to identify additional mesothelin MHC class I binding peptides if additional alleles are identified and needed to cover all subjects enrolled into this study. IFNγ ELISPOT assays will be performed to measure the frequency of cytokine-secreting T cells specific for each relevant (on the basis of HLA-restriction) mesothelin epitope using freshly thawed and enriched patient $CD8^+$ PBL as previously described(31). All peptides will be synthesized by the Johns Hopkins Peptide Core facility and will be purified to greater than 95% purity. T2 cells are TAP-deficient, human B and T lymphoblast hybrid cells, and therefore fail to transport newly processed HLA class I binding epitopes from the cytosol into the endoplasmic reticulum where these epitopes would normally bind to nascent HLA molecules and stabilize them for expression on the cell surface. T2 cells naturally express HLA-A2, but T2 cell lines have also been engineered to express additional HLA alleles, including HLA-A1, A3, A24, A11, A25, A26, A31 and A66. T2 cells expressing the appropriately matched HLA molecules will be pulsed with peptides (2 μg/ml) and used as APCs in the ELISPOT assays. Developed plates will be counted using an automated image system ELISPOT reader. The CEF pool of epitopes derived from CMV, EBV, and Influenza which is obtained from the NIH AIDS Consortium and reliably results in positive T cell responses in pancreatic cancer patients, will be used as a positive control. HIV (32) renal cell carcinoma (33) or melanoma epitopes (34) are used as negative controls. Background reactivity, measured against the negative control peptides, will be subtracted from experimental values. All time points will be assayed in a minimum of three replicates. Individual patient responses will be reported as the mean number of peptide-specific $CD8^+$ T cells per $1x10^6$ total $CD8^+$ T cells, whereas group responses will be reported as medians.

Mesothelin-specific T cell repertoire analysis. The induction of T cell responses to multiple mesothelin epitopes will be evaluated using the ELISPOT assay as described above. The induction of T cell responses to each individual epitope will be defined as a 2-fold or greater increase in the number of mesothelin epitope-specific $CD8^+$ T cells in post-vaccinated PBL as compared with that in PBL isolated prior to the first vaccination. For PBL at each post-vaccinated time point, the mesothelin-specific T cell repertoire is defined by the number of different mesothelin epitopes for which an induced response is

measured. The size of the T cell repertoire is thus the total number or percentage of epitopes for which an induction is measured.

Mesothelin-specific T cell avidity analysis. Dilutional tetramer staining will be performed on unmanipulated PBL isolated prior to and following immunotherapy as previously described(11). HLA class I tetramers are manufactured by Beckman Coulter under quality controlled conditions. Based on prior unpublished studies assessing T cell avidity in resected patients who received the GM-CSF-secreting vaccine, staining will be performed at tetramer dilutions ranging from 1:10 to 1:500 starting with 1 μM tetramer stock solutions. Flow cytometry will be used to determine the greatest dilution at which tetramer staining is lost. Higher avidity is associated with detectable staining at lower tetramer concentrations. Background staining will be assessed using tetramers prepared with irrelevant peptides. This analysis will be limited to the subset of patients for which tetramers are available. Tetramers are currently available for each of the 6 HLA-A0201-binding mesothelin epitopes and under development for the 8 HLA-A0101 epitopes.

11.0 Drug Formulation, Availability, And Preparation

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

Discard unused portions of injectable chemotherapeutic agents that do not contain a bacteriostatic agent or are prepared with unpreserved diluents (i.e., Sterile Water for Injection USP or 0.9% Sodium Chloride for Injection USP) within eight hours of vial entry to minimize the risk of bacterial contamination.

The total administered dose of chemotherapy may be rounded up or down within a range of 5% of the actual calculated dose. BSA will be calculated at baseline and prior to each cycle.

11.1Cyclophosphamide

Other names
Cytoxan. Neosar. The chemical name for cyclophosphamide is 2-[bis(2-chloroethyl)amino]tetrahydro-2H-1,3,2-oxazaphosphorine 2-oxide monohydrate.

Classification
Alkylating agent. Antineoplastic, immunosuppressant.

Mode of action
Cyclophosphamide is classed as an alkylating agent of the nitrogen mustard type. An activated form of cyclophosphamide, phosphoramide mustard, alkylates or binds with many intracellular molecular structures, including nucleic acids. Its cytotoxic action is primarily due to cross-linking of strands of DNA and RNA, as well as to inhibition of protein synthesis. Metabolism is primarily hepatic via CYP450. Excretion is in the urine (5-25% unchanged). Half-life 3- 12 hours.

Storage and stability
Cyclophosphamide for Injection, USP is a sterile white powder containing cyclophosphamide monohydrate. Cyclophosphamide is a synthetic antineoplastic drug chemically related to the nitrogen mustards. Cyclophosphamide is a white crystalline powder with the molecular formula C7H15CI2N2O2P•H2O and a molecular weight of 279.1. Cyclophosphamide is soluble in water, saline, or ethanol and has the following structural formula:

Dose Specifics

When used as the only oncolytic drug therapy, the initial course of cyclophosphamide for patients with no hematologic deficiency usually consists of 40 to 50 mg/kg given intravenously in divided doses over a period of 2 to 5 days. Other intravenous regimens include 10 to 15 mg/kg given every 7 to 10 days or 3 to 5 mg/kg twice weekly. Oral cyclophosphamide dosing is usually in the range of 1 to 5 mg/kg/day for both initial and maintenance dosing. Many other regimens of intravenous and oral cyclophosphamide have been reported.

Preparation

Cyclophosphamide should be prepared for parenteral use by infusion by adding Sterile Water for Injection, USP. Cyclophosphamide, constituted in water, is hypotonic and should not be injected directly. Add the diluent to the vial and shake it vigorously to dissolve. If the powder fails to dissolve immediately and completely, it is advisable to allow the vial to stand for a few minutes. Store intact vials of powder at room temperature of 15°C to 30°C (59°F to 86°F). Reconstituted solutions are stable for 24 hours at room temperature and 6 days under refrigeration 2°C to 8°C (36°F to 46°F). Further dilutions in D_5W or NS are stable for 24 hours at room temperature (25°C) and 6 days at refrigeration.

Administration

IV infusions may be administered over 1-24 hours. Doses of up to 2 gm may be administered over 20- 30 mins. To minimize bladder toxicity, increase fluid intake before and after cyclophosphamide administration. High dose cyclophosphamide should be accompanied by vigorous hydration and MESNA.

Compatibilities

Stable in D_5LR, D_5NS, D_5W, LR, $^1/_2NS$, or normal saline.

Drug interactions

The rate of metabolism and the leukopenic activity of cyclophosphamide are increased by chronic administration of high doses of phenobarbital. Cyclophosphamide treatment, which causes a marked and persistent inhibition of cholinesterase activity, potentiates the effect of succinylcholine chloride. Interactions with allopurinol, cardiac glycosides, CYP inducers/inhibitors.

Availability

Cyclophosphamide for Injection, USP contains cyclophosphamide monohydrate and is supplied in vials for single dose use of 500 mg, 1.0 g or 2.0 g size. Tablet forms, 25 mg or 50 mg.

Side Effects

Carcinogenesis, Mutagenesis, Fertility Impairment: Second malignancies have developed in some patients treated with cyclophosphamide used alone or in association with other antineoplastic drugs. Cyclophosphamide can cause fetal harm when administered to a pregnant woman. Cyclophosphamide interferes with oogenesis and spermatogenesis. It may cause sterility in both sexes. Development of sterility appears to depend on the dose of cyclophosphamide, duration of therapy, and the state of gonadal function at the time of treatment. Amenorrhea associated with decreased estrogen and increased gonadotropin secretion can occur.

GI: Nausea, vomiting, diarrhea, stomatitis.

Endocrine/Metabolic: Hyponatraemia, SIADH, transient succinylcholinesterase deficiency.

Cutaneous: Rash, alopecia.

Pulmonary/ENT: Interstitial pneumonitis. Nasal congestion when IV doses administered too rapidly. Occasionally, runny eyes, sinus congestion and sneezing.

Cardiac: A few instances of cardiac dysfunction have been reported following use of recommended doses of cyclophosphamide. Acute cardiac toxicity has been reported with doses as low as 2.4 g/m^2 to as high as 26 g/m^2. Rarely high doses of cyclophosphamide, severe, and sometimes fatal, congestive heart failure has occurred after the first cyclophosphamide dose (haemorrhagic myocarditis). Cyclophosphamide has been reported to potentiate doxorubicin-induced cardiotoxicity.
Hematologic: Anemia, neutropenia, thrombocytopenia.

Infections: Treatment with cyclophosphamide may cause significant suppression of immune responses. Serious, sometimes fatal, infections may develop in severely immunosuppressed patients.

Renal/GU: Hemorrhagic cystitis may develop in patients treated with cyclophosphamide. Fibrosis of the urinary bladder, sometimes extensive, also may develop with or without accompanying cystitis. Such bladder injury is thought to be due to cyclophosphamide metabolites excreted in the urine. Forced fluid intake helps to assure an ample output of urine, necessitates frequent voiding, and reduces the time the drug remains in the bladder. Hematuria usually resolves in a few days after cyclophosphamide treatment is stopped, but it may persist.

Nursing implications
Similar in name to several cytotoxics, e.g., cisplatin, cytarabine, Cytosar, Cytotec.

11.2Whole Cell Vaccine

Other names
Lethally Irradiated Allogeneic Granulocyte-Macrophage Colony-Stimulating Factor Secreting Tumor Vaccine, GVAX.

Classification
Whole cell vaccine. Immunotherapy.

Manufacture and Supply
The whole cell vaccine is prepared and supplied by Cell Processing and Gene Therapy Facility (CPGT) at Johns Hopkins University.

[redacted]

Mode of Action
Immunologic.

Storage and Stability
Each whole cell vaccine vial is shipped frozen and stored frozen in vapor phase liquid nitrogen in the injectable formulation.

At the time of vaccination, the vials are removed from the appropriate storage conditions and quickly thawed in a 37°C water bath. The thawed vials are transferred from the

water bath to wet ice until the vaccine is drawn from the vial with a 16 gauge needle into 1 cc luer lock syringes and then changed to 22 gauge luer lock needles for subject's administration. The syringes are then released to the appropriate medical personnel for intradermal injection and are kept on ice until the vaccine is administered. All injections must be given within 3 hours of thaw. The 3 hours start when the vials are placed in the water bath for thaw.

Dose Specifics
Each vaccine will consist of 6 intradermal injections. Each injection consists of approximately 8.3 $x10^7$ cells in 0.7 to 0.73 ml solution.

Administration
Intradermal injection. Each vaccination will consist of a total of six intradermal injections, two each in the upper right and left thighs, and two in the upper non-dominant arm. A wheal should be created. See **Appendix I** for further details.

Incompatibilities
No information available.

Side Effects
Dermatologic: Erythema, induration, pain/soreness, at vaccine site, lasting for up to a week. Pruritus, blister formation, hyperpigmentation, rash.

Hematologic: Eosinophilia, leukocytosis.

Other: Flu-like symptoms, fatigue, fever, chills, myalgias.

Immunologic: Autoimmunity (theoretic).

Disposal
The vaccine is considered a biosafety hazard. All used vials and all material that comes in contact with the vaccine should be disposed of in a biosafety hazard container (e.g., Sharp's container or red bag) and then treated as medical waste in accordance with institution practice. Each institution should abide by federal, state and local waste disposal regulations. Material from the vaccine vials should not be poured or pipetted into a drain for disposal to sanitary sewer systems.

Nursing Implications
Each vaccine consists of a total of 6 intradermal injections. Two each in the upper right and left thighs and two in the upper non-dominant arm. Lidocaine-based topic anesthetic (EMLA or Lidocaine) will be applied to each injection site at least 1 hour prior to vaccination and covered with an occlusive dressing. Do not shave the sites prior to applying the numbing cream. Omit Lidocaine if subject is allergic. For upper extremity injection, use the upper portion of the arm. For lower extremity injection, use the upper portion of the thigh. Injections can be placed randomly, but at least 2 inches apart. If the patient is unable to receive an injection in the arms or legs, an acceptable alternative injection site is the abdomen. Do not place injections into skin that is ulcerated or infected. Topical steroids and topical antihistamines should be avoided. Each dose will be administered on an outpatient basis. Patients must remain in the clinic for observation for at least 30 minutes following the vaccination. See **Appendix I** for further details.

12.0 Ancillary Therapy

12.1 Patients should receive full supportive care, including transfusions of blood and blood products, erythropoetin (unless otherwise specified in the protocol), antibiotics, antiemetics, etc., when appropriate.

12.2 Treatment with hormones or other chemotherapeutic agents may not be administered except for steroids given for adrenal failure; hormones administered for non-disease-related conditions (e.g., insulin for diabetes); and intermittent use of dexamethasone as an antiemetic in solid tumor protocols. Megesterl acetate (Megace) is permitted to support anorexia.

13.0 CRITERIA FOR RESPONSE, PROGRESSION AND RELAPSE (SOLID TUMORS)

For the purposes of this study, patients should be reevaluated per study calendar and per standard of care as described in section 7.0 after completion of adjuvant therapy until taken off study.

Most patients will be expected to have only minimal residual microscopic disease if they remain to be eligible for this study following the surgery. Therefore, there will be no disease to measure at baseline. Patients will be monitored for disease-free and overall survival. The results from the current trial will be compared to the results of the prior phase II adjuvant study conducted at Johns Hopkins Hospital, conducted with a similar treatment protocol.

Patients will undergo standard of care evaluations consisting of abdominal, chest, and pelvis CT scans at regular intervals to evaluate for local recurrence and distant metastases. In addition, any patient presenting with symptoms will undergo evaluation for metastases. Recurrent disease is defined as evidence of either definitive local or metastatic recurrence by CT scan. The serum tumor marker CA 19-9 lacks a sufficient sensitivity and specificity to serve as reliable indicators of response/recurrence. The CA 19-9 levels will be followed to evaluate whether large and persistent changes might correlate with either in vitro immune responses or with time to clinical recurrence.

13.1Definition of Progression

Distant progressive disease is defined as the unequivocal appearance of any lesions. This includes liver metastases, pulmonary metastases, bone metastases, brain metastases and mesenteric/abdominal nodal progression outside the radiation CTV. Malignant ascites will also be scored as distant progression if cytologic evaluation of cytology fluid yields malignant cells.

Locoregional progression is defined as progressive change from baseline post-operative CT in the context of rising CA19-9, increasing symptoms such as pain or biliary obstruction of the hepaticojejunostomy, or biopsy confirmation.

13.2Evaluation of Immune Responses

See section 10 of protocol.

14.0 Statistical Considerations

Statistical Analysis Plan

The primary endpoint is safety. The toxicity profile of FOLFIRINOX (F) in the metastatic setting and standard radiation therapy (RT) in the adjuvant setting has been well established. Approximately 20% of patients experience diarrhea, 50% experience non-recoverable neutropenia, and 10% experience thrombocytopenia. We are interested in exploring the combination of FOLFIRINOX with stereotactic body radiation therapy (SBRT) plus GVAX.

Toxicity rates of grade 3-4 diarrhea, grade 3-4 neutropenia, and grade 3-4 thrombocytopenia would be considered unacceptable (dose limiting toxicity-DLT) if they were 40%, 60%, and 40% respectively. Although GVAX is not expected to contribute to these two types of toxicity, we will enroll patients into two cohorts so that only one factor in the therapy is modified at each step. The first cohort will receive FOLFIRINOX plus SBRT in place of standard radiation therapy. The second cohort will receive the same combination plus GVAX.

A decision rule similar to the traditional 3+3 design will be used to determine whether or not it is safe to continue on to the next cohort. An initial 3 patients will be treated with F+SBRT. If no toxicities are observed within the first 2 cycles of FOLFIRINOX administration (8 weeks) then the next group of patients will be treated with F+SBRT+GVAX. If 2-3 patients are observed with uncontrolled grade 3-4 diarrhea, 2-3 patients are observed with grade 3-4 thrombocytopenia, or if 3 patients are observed with grade 3-4 neutropenia within the first 2 cycles of FOLFIRINOX administration (8 weeks) then the trial will be halted. Otherwise (i.e. if 1 patient is observed with grade 3-4 diarrhea, 1 patient is observed with thrombocytopenia, or 1-2 patients are observed with neutropenia), then an additional 3 patients will be treated with F+SBRT. If 0-1/6 patients experience grade 3-4 diarrhea, 0-1/6 patients experience grade 3-4 thrombocytopenia, and 0-3/6 experience grade 3-4 neutropenia, then the next group of patients will receive F+SBRT+GVAX. Treatment groups for which < 40% experience grade 3-4 diarrhea (i.e. < 2/6), < 40% experience grade 3-4 thrombocytopenia (i.e. <2/6), and < 60% experience grade 3-4 neutropenia (i.e. < 4/6) will be considered tolerable. An expansion cohort of 6 patients will be treated with F+SBRT+GVAX, if tolerable, in order to refine estimates of toxicity and initial efficacy for a total sample size of 15-18 patients.

-Any grade 3-4 toxicity within the context of the decision rule described above will be dose limiting with the exception of 1) nausea, vomiting or diarrhea that improves to ≤ grade 2 with maximal medical management within 5 days; 2) neutropenia that resolves within 5 days; or 3) thrombocytopenia that resolves within 7 days.

-Patients who get DLT-qualifying diarrhea but do not adhere to appropriate medical management will be considered inevaluable for DLTs and will be replaced.

The number, type and degree of toxicities will be tabulated. In particular, the toxicities for each round of vaccination will be followed and the proportion of individuals who have a DLT within the first 28 days of vaccination will be estimated with exact 95% confidence intervals. Poisson modeling with robust confidence intervals will be used to estimate the rate per individual as well as the rate per vaccine dose.

Secondary objectives: Due to the small sample size, the analysis of secondary outcomes will be primarily exploratory in nature. Overall survival (OS) is defined as the time from surgery until death. Disease free survival (DFS) is defined as the time from surgery until disease progression or death. All enrolled individuals will the included in the analysis. Individuals who are lost to follow-up will be included in the analysis and censored at the date of the last visit for DFS and date of the last contact for OS. For time-to-event outcomes (OS and DFS), Kaplan-Meier estimates of the survival function will be used to graphically compare treatments as well as subgroups of interest. Log-rank tests and Cox proportional hazards models will be used to statistically compare the two treatment arms as well as subgroups of interest.

Continuous variables will be summarized with means and standard deviations or medians with ranges, as appropriate. Dichotomous and categorical variables will be summarized using proportions with exact 95% confidence intervals and counts respectively. Linear regression and logistic regression models will be used to compare treatment arms for continuous and binary variables, respectively. In the case of repeated measurements, generalize estimating equations will be used to account for the correlation between measurements within an individual.

Additional analyses will be conducted to examine the immune response parameters for patients in the vaccine arm. Analyses will be assessed for each dose and related to clinical outcomes. The primary focus will be upon the 3 immune response parameters identified previously but additional exploratory analyses will be completed to identify novel parameters. These summaries will be computed for each patient both pre- and post- administration of each vaccination. Plots will be used to show the changes in immune response over time both for each individual and summarized for the study as a whole. For each vaccination, comparisons in the pre- and 28 day post-vaccine responses will be made using paired t-tests (or Wilxcoxon signed rank tests if appropriate) for continuous measurements. McNemar's test will be used to identify significant changes in the percentage of individuals with a dichotomous characteristic pre- and post-vaccine. Associations between immune parameters will be explored graphically (e.g. scatterplots, boxplots) and numerically (e.g. correlations, Kappas, McNemar's tests). The relationships between the immune parameters and clinical outcomes will be assessed using univariate and multivariate modeling. In the case of a binary clinical outcome (e.g. toxicity, DFS>20 months vs. DFS< 20 months years), logistic regression will be used. In the case of a time-to-event clinical outcome (e.g. OS), Cox proportional hazards models will be used.

15.0 Adverse and Problem Event Reporting

15.1 Recording of an adverse event

The principal investigator is responsible for evaluating all adverse events, obtaining supporting documents, and determining that documentation of the event is adequate. The Investigator should attempt, if possible, to establish a diagnosis based on the patient's signs and symptoms. When a diagnosis for the reported signs or symptoms is known, the Investigator should report the diagnosis, not the symptoms, as the adverse event. The principal investigator is responsible for determining the severity and relationship of the adverse event to the investigational drug. The principal investigator may delegate these duties to sub-investigators and must assure that these sub-investigators are qualified to perform these duties under the supervision of the principal investigator.

The Investigator or designee must completely and promptly record each adverse event using the eCRF, regardless of relationship to study drug as determined by the Investigator. All adverse events will be recorded in the subject's Case Report Form and in the study data base. The detailed description of the event will include appropriately graded severity of the adverse event and its relationship to the study drug.

Severity will be categorized by toxicity grade according to the NCI Common Terminology Criteria for Adverse Events version 4.0 available at http://ctep.cancer.gov/reporting/ctc.html

Adverse events not listed in the NCI Common Terminology Criteria for Adverse Events will be evaluated using the following criteria:

- Grade 1, Mild: Awareness of symptom, but easily tolerated; usually transient requiring no special treatment; does not interfere with usual status or activities
- Grade 2, Moderate*:* May be ameliorated by simple therapeutic measures; may interfere with usual activities
- Grade 3, Severe*:* Incapacitating, inability to perform usual activities
- Grade 4, Life-threatening/Disabling*:* Subject was at risk of death or significant disability at the time of the event
- Grade 5, Death related to AE

Relationship of the adverse event to the investigational drug will be determined by the principal investigator, and will be categorized as:

- ***Not Related:*** The adverse event is clearly related to other factors such as the subject's clinical state, environmental factors, or other modes of therapy or concomitant drugs administered to the subject.
- ***Not likely*****:** There is a temporal relationship to investigational product administration, but there is not a reasonable causal relationship between the investigational product and the AE.
- ***Possible:*** The adverse event follows a reasonable temporal sequence from administration of the study drug, and/or follows a known response pattern to the study drug, but could readily have been produced by the subject's clinical state, environmental factors, or other modes of therapy or concomitant drugs administered to the subject.
- ***Probable:*** The adverse event follows a reasonable temporal sequence from administration of the study drug and follows a known response pattern to the study drug, and cannot readily have been produced by the subject's clinical state, environmental factors, or other modes of therapy or concomitant drugs administered to the subject.
- ***Definite*****:** There is a reasonable causal relationship between the investigational product and the AE. The event responds to withdrawal of investigational product (dechallenge), and recurs with rechallenge when clinically feasible.

Clinically significant laboratory abnormalities present at the Baseline visit will be recorded as pre-treatment signs and symptoms. After study treatment administration, all grade 3 and 4 clinical laboratory results that represent an increase in severity from baseline will be reported as adverse events. A grade 1 or 2 clinical laboratory abnormality should be reported as an adverse event only if it is considered clinically significant by the investigator. Laboratory abnormalities will not be recorded as adverse events unless considered clinically significant by the Investigator, and clinically significant laboratory abnormalities will not be recorded as serious AEs unless the event meets the definition of serious.

In the event of death, the cause of death should be recorded as the adverse event. An attempt will be made to obtain a copy of the death certificate. Because the long-term effects of gene therapy are not known, the National Institutes of Health (NIH) would like an autopsy, in the event of death. If an autopsy is performed, a copy of the autopsy report should be obtained.

15.2Reporting guidelines

We will use the current JHM IRB, Institutional Bio-safety Committee (IBC), NIH Recombinant DNA Advisory Committee (RAC), FDA guidelines and SAE Reporting Criteria, and Safety Reporting Requirements for IND Holders for reporting relevant problems, events, adverse events, and adverse drug reactions that occur after starting experimental treatment.

ALL serious adverse events that occur after starting experimental treatment, regardless of causality, should be reported to ALL of the following:

1. IND Sponsor
2. Institution IRB
3. Institution IBC

Timelines for reporting SAE's

A. IND Sponsor (Dr. Jaffee)- within 24 hours

B. IRB- within 3 days if the SAE is related to the study drug. If the SAE is not considered related to the study drug within 10 days.

Johns Hopkins Medicine IRB

[redacted]

[redacted]

C. IBC- within 15 days

D. FDA- As per section 16.2

E. RAC- As per section 16.2

Follow up on SAE reports should also be sent to all the above agencies within two weeks of receipt of information at the site.

15.2.1 Safety Reporting Requirements for IND Holders

For **Investigator Sponsored IND Studies**, there are some additional reporting requirements for the FDA in accordance with the guidance set forth in 21 CFR 312.32. Sponsor-investigators of studies conducted under an IND must comply with the following safety reporting requirements:

15.2.1.1 Expedited IND Safety Reports:

7 Calendar-Day Telephone or Fax Report:
The Sponsor-Investigator is required to notify the FDA and RAC of any fatal or life-threatening adverse event that is **unexpected and assessed by the investigator to be possibly related to the vaccine**. An unexpected adverse event is one that is not already described in the Investigator Brochure. Such reports are to be telephoned or faxed to the FDA within 7 calendar days of first learning of the event. The RAC will be notified by e-mail within 7 calendar days of first learning of the event. Each telephone call or fax transmission (see fax number below) should be directed to the FDA new drug review division in the Center for Drug Evaluation and Research or in the product review division for the Center for Biologics Evaluation and Research, whichever is responsible for the review of the IND.

15 Calendar-Day Written Report:
The Sponsor-Investigator is also required to notify the FDA, RAC, and all participating investigators, in a written IND Safety Report, of any serious, unexpected AE that is considered possibly related to the vaccine. An unexpected adverse event is one that is not already described in the Investigator Brochure.

Written IND Safety Reports should include an Analysis of Similar Events in accordance with regulation 21 CFR § 312.32. All safety reports previously filed with the IND concerning similar events should be analyzed. The new report should contain comments on the significance of the new event in light of the previous, similar reports.

Written IND safety reports with Analysis of Similar Events are to be submitted to the FDA, RAC, and all participating investigators within 15 calendar days of first learning of the event. In this study electronic CRF will be used.

FDA fax number for IND Safety Reports:
301-827-9796

15.2.1.2 IND Annual Reports

In accordance with the regulation 21 CFR § 312.32, the Sponsor-Investigator shall within 60 days of the anniversary date that the IND went into effect submit a brief report of the progress of the investigation. Please refer to Code of Federal Regulations, 21 CFR § 312.32 for a list of the elements required for the annual report. All IND annual reports will be submitted to the FDA by the Sponsor-Investigator.

15.3Special considerations for AEs that occur during local FOLFIRINOX treatment

FOLFIRINOX has significant and well-documented toxicities and is standard of care for metastatic pancreatic adenocarcinoma. Sufficient safety data will have been collected during the first 3 cycles of FOLFIRINOX to permit local treatment for cycles 4-6.

Therefore, during any period of local FOLFIRINOX treatment, the study will be focused on monitoring and reporting:
1. All grade III and grade IV gastrointestinal adverse events
2. All toxicities deemed by the study PI as related to SBRT, Cyclophosphamide or GVAX
3. All SAEs

These events will be recorded as described in Section 15.1 and their severities will still be categorized by NCI CTCAE v4.0 criteria. Relationship of these events to the investigational drug will be determined by the principal investigator together with oncology co-investigators of the study team. Reporting of these events will follow the same guidelines described in Section 15.2

16.0 Clinical Trial Monitoring

The SKCCC Compliance Monitoring Program will provide external monitoring for JHU-affiliated sites in accordance with SKCCC DSMP (Version 6.0, 02/21/2019). The SMC Subcommittee will determine the level of patient safety risk and level/frequency of monitoring. The Principal Investigator is responsible for internal monitoring for both safety and data quality. Data monitoring of this protocol will occur on a regular basis with the frequency dependent on the rate of subject accrual and the progress of the study. On a regular basis the protocol will be internally monitored by the principal investigator, and the study's sponsor, Dr. Elizabeth Jaffee.

16.1 Internal review

The PI will have a regular internal monitoring.

The study will be monitored internally by the Principal Investigator. The internal review should occur after the first three patients are enrolled and have completed two cycles of therapy. The process should occur every six months thereafter. The PI will review data to assure the validity of data, as well as, the safety of the subjects. The PI will also monitor the progress of the trial. The PI will review safety reports and clinical trial efficacy endpoints and to confirm that the safety outcomes favor continuation of the study.

The PI will be responsible for maintaining the clinical protocol, reporting adverse events, assuring that consent is obtained and documented, reporting of unexpected outcomes, and reporting the status of the trial in the annual report submitted to the IRB. Content of the report will include year-to-date and full trial data on: accrual and eligibility, protocol compliance, treatment administration, toxicity and adverse events, response, survival, regulatory compliance, compliance with prearranged statistical goals. The following will be reviewed:

- Original, signed consent forms to ensure that one is available for each subject.

- Case report forms and source documentation for validity and consistency in the completed entries, as well as, accuracy, legibility, signatures and dates.

- Treatment administration records to ensure concurrence between dispensing records and CRFs as to subject identity, and dosage of study drug administered.

- Compliance with the protocol will also be checked.

- Pharmacy drug accountability records for accuracy and completeness, and study drug storage to ensure proper maintenance and supply levels.

16.2 External review

External data monitoring will be performed by the SKCCC at Johns Hopkins Clinical Research Office Quality Assurance Program (CRO QA) in accordance with SKCCC guidelines. Data and safety monitoring oversight will be conducted by the SKCCC at Johns Hopkins Safety Monitoring Committee (SMC). Per the SKCCC at Johns Hopkins Safety Monitoring Plan, the CRO QA will forward summaries of all monitoring reports to the medical expert committee (MEC) and JHU SKCCC Safety Monitoring Committee for review. All reportable anticipated and unanticipated protocol events/problems and amendments that are submitted to the IRB will also be reviewed by the JHU SKCCC Safety Monitoring Committee Chair (or designee) and QA manager.

The MEC will review safety data on at least a semi-annual basis. The MEC will provide a written summary of each assessment to the Principal Investigator after each meeting. In turn, the study team will forward these summaries to the JHU IRB and JHU SKCCC SMC. The operating plan of the MEC will be as follows:

- Meetings will be held at least semi-annually, and potentially more frequently if needed.
- Meetings will be conducted in-person or via video/teleconference, with a participant sign-in sheet collected at each meeting.
- Approximately one week prior to each MEC meeting, the study team will submit the following items to MEC personnel for review and discussion at the meeting:
    - A summary of the clinical trial's progress to date;
    - The latest IRB-approved consent document;
    - A summary of all AEs, SAEs, deaths, and withdrawals to date

Note that the MEC reserves the right to halt trial accrual or all study activity if, after review, serious safety concerns warrant this action. If the MEC halts study accrual or all study activity, then the study team must notify the JHU SKCCC SMC, JHU IRB, FDA, and NIH RAC immediately.

The MEC consists of the following members:

- Dr Russel Hales (Radiation oncology); [redacted]
- Dr Chuck Drake (Medical oncology); phone: [redacted]
- Dr Christine Hahn (Medical oncology); [redacted]

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Appendix I: Vaccine Administration Standard Operating Procedure

Purpose: To describe the standard procedure administering the pancreatic vaccine

Scope: Licensed personnel (Registered Nurse or Nurse Practitioner) administering the pancreatic vaccine.

Procedure:

Supplies

1. Obtain supplies: wipes, alcohol pads and band aids

Patient preparation

1. Assess patient and hold vaccination for fevers, flu-like symptoms, and any other worrisome symptoms.
2. Apply lidocaine based numbing cream (omit if pt is allergic) to anticipated vaccine sites 1-2 hours prior to vaccination and cover with an occlusive transparent dressing. Two sites on the non-dominant upper arm, two sites on the right and left upper thighs. (**refer to SOP**)

Patient Administration

1. After validating correct patient and correct vaccine (**refer to SOP**), remove occlusive dressings one at a time and remove excess numbing cream with a clean dry wipe.
2. Cleanse site with alcohol pad and let air dry.
3. Assess color and consistency of vaccine solution.
4. Remove all air from syringe barrel.
5. Administer vaccine syringe, bevel up, intradermally slowly over 30-60 seconds to reduce discomfort and create a wheal.
6. Place band aid over site.
7. Monitor patient for at least 30 minutes after administration of the vaccine.

Purpose: To describe the standard procedure for validating correct patient and correct vaccine prior to administration.

Scope: Licensed personnel (Registered Nurse or Nurse Practitioner) administering the vaccine.

Procedure:

1. Obtain order form and patient's identification card (ie: hospital patient identifier card)

2. Administering RN or NP and vaccine preparation personnel confirm the following against the order form:
    a. Patient name
    b. Date of birth/hospital ID card number
    c. Vaccine lot numbers
    d. Thaw time
    e. Quantity of each syringe
    f. Expiration time

3. Keep all vaccine syringes on ice until ready to administer.

Purpose: To describe the standard procedure for self or study personnel application of the lidocaine-based numbing cream.

Scope: Subjects self applying lidocaine-based numbing cream and study personnel applying cream.

Supplies: lidocaine-based numbing cream and (6) occlusive dressings

Procedure:

1. Obtain numbing cream and (6) occlusive dressings.
2. Wash hands before applying the lidocaine-based cream.
3. The lidocaine-based cream should be placed on the non-dominant upper arm and both upper thighs in 2 areas.
4. Clean off arm and thighs sites with soapy water and dry thoroughly.
5. Open the occlusive transparent dressings (6) and place beside you.
6. Put a quarter size dollop of the lidocaine-based cream on your non-dominant upper arm (below the shoulder in the front of the arm) and cover with the occlusive dressing.
7. Put another quarter size dollop of the lidocaine-based cream on the same arm about 4 inches below the other area and just to the side and cover with the occlusive dressing.
8. Now do the same for both of the thigh sites. Place a quarter size dollop of the lidocaine-based cream on the upper thigh (about 4-5 inches below the underwear line) and cover with the occlusive dressing.
9. Place another dollop of the lidocaine-based cream on your inner thigh site about 5 inches below the other dressing.
10. Repeat both #8 and #9 on the other thigh.
11. There should be a total of 6 sites with the lidocaine-based cream covered with the dressings.
12. Once finished, wash hands with warm soapy water to remove any excess cream.
13. You may dress, but do not cross legs or place anything on lap since this may have the cream leak out from under the dressing.

PLEASE AVOID ANY EYE CONTACT WITH THE CREAM. THIS CREAM IS A LIDOCAINE-BASED NUMBING CREAM AND COULD CAUSE EYE IRRITATION. IF EYE CONTACT IS MADE, WASH EYE WITH WARM WATER FOR 10 MINUTES TO FLUSH THE CREAM FROM THE INNER EYE.

Appendix II: Patient Documentation for Vaccination

Pt name:_____ Date:_____

Vaccine #:____ Thaw time: _____

Topical anesthetic applied at: _____Pt is R or L handed

Right/Left upper arm: Amount: Time:
(use nondominant arm unless otherwise indicated)

Proximal site _____ _____

Distal site _____ _____

Right/Left upper thigh: Amount: Time:

Proximal site _____ _____

Distal site _____ _____

Right/Left upper thigh: Amount: Time:

Proximal site _____ _____

Distal site _____ _____

Assess previous vaccination reactions:

Redness: start: resolved:
Tenderness: start: resolved:
Induration: start: resolved:
Pruritis: start: resolved:
Other symptoms: start: resolved:

Vital signs:

Medication Changes:

Appendix III: Oxaliplatin Prescribing Information

Appendix IV: 5-Fluorouracil Prescribing Information

Appendix V: Leucovorin Prescribing Information

Appendix VI: Irinotecan Prescribing Information

Appendix VII: Serious Adverse Event Reporting Form

Serious Adverse Event Reporting Form

Please notify Dr. Jaffe and Dr. Laheru within 24 hour

Protocol Title:	**A Phase II Study Evaluating Allogeneic Pancreatic Tumor Immunotherapy, Fractionated Stereotactic Body Radiation Therapy (SBRT) and FOLFIRINOX chemotherapy in Patients with Resected Adenocarcinoma of the Pancreas**		
Protocol Number:	**Principal Investigator:**	**Signature of PI:**	**Date:**
Report Date:	**Hospital Admission Date:**	**Date of Discovery of Event:**	**Report Type:** ☐ **Initial** ☐ **Follow-up** ☐ **Final Follow-up** ☐ **Death** ☐ **Addendum to:**

Section A: Subject Information

Subject ID:	**Subject Initial:**	**Subject Gender:** ☐ **Male** ☐ **Female**

Section B: Event Information

Event diagnosis or symptoms:	**Date of First Dose (vaccine):** **Date of Last Dose (vaccine) prior to Event:** **Number of Total Doses (vaccine):**	**Action taken with the study drug (Cyclophosphamide):** ☐ **None** ☐ **Interrupted** ☐ **Discontinued**	**Action taken with the study drug (vaccine):** ☐ **None** ☐ **Interrupted** ☐ **Discontinued**
Event Onset Date:		**Event End Date:**	

Relationship to:	**Cyclophosphamide**	**Vaccine**	**Chemotherapy**	**SBRT**	**Underlying Disease**
Unrelated	☐	☐	☐	☐	☐
Probably Unrelated	☐	☐	☐	☐	☐
Possible Related	☐	☐	☐	☐	☐
Probably Related	☐	☐	☐	☐	☐
Definitely Related	☐	☐	☐	☐	☐

Section C: Brief Description of the Event:
Section D: Relevant Medical History
Section E: Concomitant Drug (Not related to SAE)

Name of the Drug	**Start Date**	**Stop Date**	**Route**	**Dose**	**Frequency**

Section F: Comments
Additional Documents: ☐ Please specify