A safety and feasibility trial of boost vaccinations of a lethally irradiated, allogeneic pancreatic tumor cell vaccine transfected with the GM-CSF gene given alone or in combination with either a single intravenous dose or daily metronomic oral doses of cyclophosphamide for the treatment of surgically resected pancreatic adenocarcinoma

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Study Synopsis

Title: A safety and feasibility trial of boost vaccinations of a lethally irradiated, allogeneic pancreatic tumor cell vaccine transfected with the GM-CSF gene given alone or in combination with either a single intravenous dose or daily metronomic oral doses of cyclophosphamide for the treatment of surgically resected pancreatic adenocarcinoma

Objectives:

Primary:
To evaluate the safety and feasibility of long term boost vaccinations of a lethally irradiated, allogeneic pancreatic tumor cell vaccine transfected with the GM-CSF gene given 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 head, neck, or uncinate process of the pancreas.

Secondary:
1. To assess the effect of boost vaccinations and long-term treatment of immune modulating doses of cyclophosphamide on the number, repertoire and avidity of peripheral mesothelin-specific $CD8^+$ T cells.
2. To estimate disease-free and overall survival of surgically resected pancreatic adenocarcinoma patients treated with vaccine boosts with or without low dose cyclophosphamide.

Study Population:
In order to be considered for this study, patients need to meet the following criteria for inclusion:
- History of surgically resected pathologic stage 1 (no direct tumor extension beyond pancreas and no regional lymph node metastases), 2a (direct extension of tumor beyond pancreas), and/or 2b (regional lymph node metastases) adenocarcinoma of the head, neck, tail, or uncinate of the pancreas.
    - **Cohort 1**: Patients who have previously participated in the study titled "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" [J0810, NA_00015858 (formerly 00-01-58-58)], have no radiographic evidence of pancreatic disease recurrence, and have received the GM-CSF secreting allogeneic pancreatic cancer vaccine.
    - **Cohort 2**: Patients who never received any type of pancreatic vaccine or immunotherapy, had the Whipple surgery within 18 months and completed the planned adjuvant chemotherapy and/or chemoradiation. Have no radiographic evidence of pancreatic cancer disease recurrence. Have not received any anti-cancer therapy in the past 28 days.
    - **Cohort 3**: Patients who have previously participated in the study titled "A Randomized Study of a GM-CSF secreting allogeneic pancreatic cancer vaccine with or without a PD-1 Blockade Antibody (Nivolumab) for the Neoadjuvant and Adjuvant Treatment of Patients with Surgically Resectable Adenocarcinoma of the Pancreas" (IRB00050517,

J1568), have no radiographic evidence of pancreatic disease recurrence, and received their sixth vaccine within 12 months of enrolling in J09100.

- **Cohort 4**: Patients who have previously participated in the study titled "A Phase II Study of GM-CSF secreting allogeneic pancreatic cancer vaccine in combination with PD-1 Blockade Antibody (Pembrolizumab) and Stereotactic Body Radiation Therapy (SBRT) for the Treatment of Patients with Locally Advanced Adenocarcinoma of the Pancreas" (IRB00083132, J15237), have no radiographic evidence of pancreatic disease recurrence, and received their last vaccine in the extended treatment phase within 12 months of enrolling in J09100.
- **Cohort 5**: Patients who have previously participated in the study titled "A Pilot Study of a GVAX Pancreas Vaccine (with Cyclophosphamide) in Combination with a PD-1 Blockade Antibody (Pembrolizumab) and a Macrophage Targeting Agent (CSF1R inhibitor) for the Treatment of Patients with Borderline Resectable Adenocarcinoma of the Pancreas" (IRB00130267, J1766), have no radiographic evidence of pancreatic disease recurrence, and received their last vaccine in the extended treatment phase within 12 months of enrolling in J09100.

Study Design:
Eligible subjects will receive by intradermal administration the pancreatic tumor vaccine consisting of two irradiated, allogeneic pancreatic tumor cell lines transfected with the granulocyte macrophage-colony stimulating factor (GM-CSF) gene with or without low dose cyclophosphamide. Study participants will be recruited from our prior neoadjuvant vaccination with or without low dose cyclophosphamide trial and vaccine naïve patients. The vaccination boosts will be offered as a continuation of care.

Patients from the J0810 study (Cohort 1) will remain on the same arm as the J0810 study where they have received the parental vaccine (**Figure 1**). The first vaccine boost will be given no sooner than six months (+/- 1 month) after the last prime vaccination. The vaccine will be administered for all arms once every six months (+/- 1 month) after the previous vaccine until five years have passed and then once every 12 months (+/- 1 month) until 10 years have passed, the subject no longer meets the eligibility criteria, no longer wishes to participate in the study, or the vaccine supply is exhausted. Arm A participants will receive the pancreatic cancer vaccine alone. Arm B participants will be vaccinated and receive a single low-dose of cyclophosphamide (200 mg/m^2) intravenously one day prior to vaccination (**Figure 2**). Participants in Arm C will receive cyclophosphamide 50 mg once a day starting from 28 days prior to day 1 of vaccination until 28 days post vaccination. We estimate that approximately 20 patients from the J0810 study will be candidates for this boost vaccination study.

Vaccine naïve patients (Cohort 2) will first receive three prime vaccines each one month apart and each in combination with a single low-dose of cyclophosphamide (200 mg/m^2) intravenously one day prior to vaccination (**Figure 1**). Then, they will receive the boost vaccines as the participant in Arm B from the J0810 study (**Figure 2**). Patients from Cohort 2 may receive treatment until ten years have passed, the subject no longer meets the eligibility criteria, no longer wishes to participate in the study, or the vaccine supply is exhausted. We estimate the sample size of the vaccine-naïve cohort to be 52.

Participants from clinical trials J1568, J15237 and J1766 (Cohorts 3, 4 and 5) will be vaccinated and receive a single low-dose of cyclophosphamide (200 mg/m^2) intravenously one day prior to vaccination (**Figure 2**). The first boost vaccine will be given no sooner than six months (+/- 1 month) and within 12 months after the last prime vaccination on the primary study. The vaccine will be administered once every six months (+/- 1 month) after the previous vaccine until five years have passed, the subject no longer meets the eligibility criteria, no longer wishes to participate in the study, or the vaccine supply is exhausted. We estimate the sample size of Cohort 3, 4 and 5 to be 68.

Study Drug:
The vaccine consists of equal numbers (2.5 x 10^8 each) of Panc 6.03 and Panc 10.05 cells combined into a single vaccination. Vaccine cells from each pancreas tumor cell line frozen at 1.25 x10^8 cells/vial (2 vials per cell line) in an injectable formulation of hetastarch will be thawed on the day of vaccination and taken up into syringes. Each vaccination will consist of six total intradermal injections, two each in the right and left thighs, and two in the non-dominant arm. This preparation has been used in two completed phase II studies and in two on-going phase II studies. Cyclophosphamide (Cytoxan, CTX) is given at 50 mg once daily per oral route or 200 mg/m^2 intravenously as indicated in the study design.

1.0 Study Schema

Figure 1 Vaccination Schedule for Cohorts 1-5

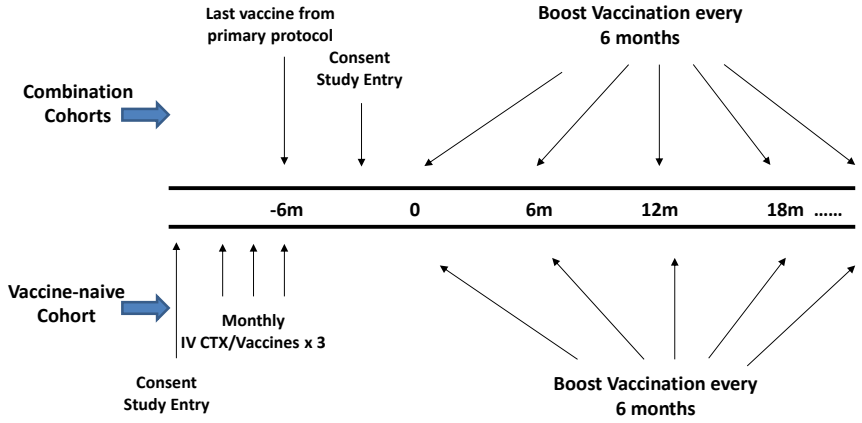
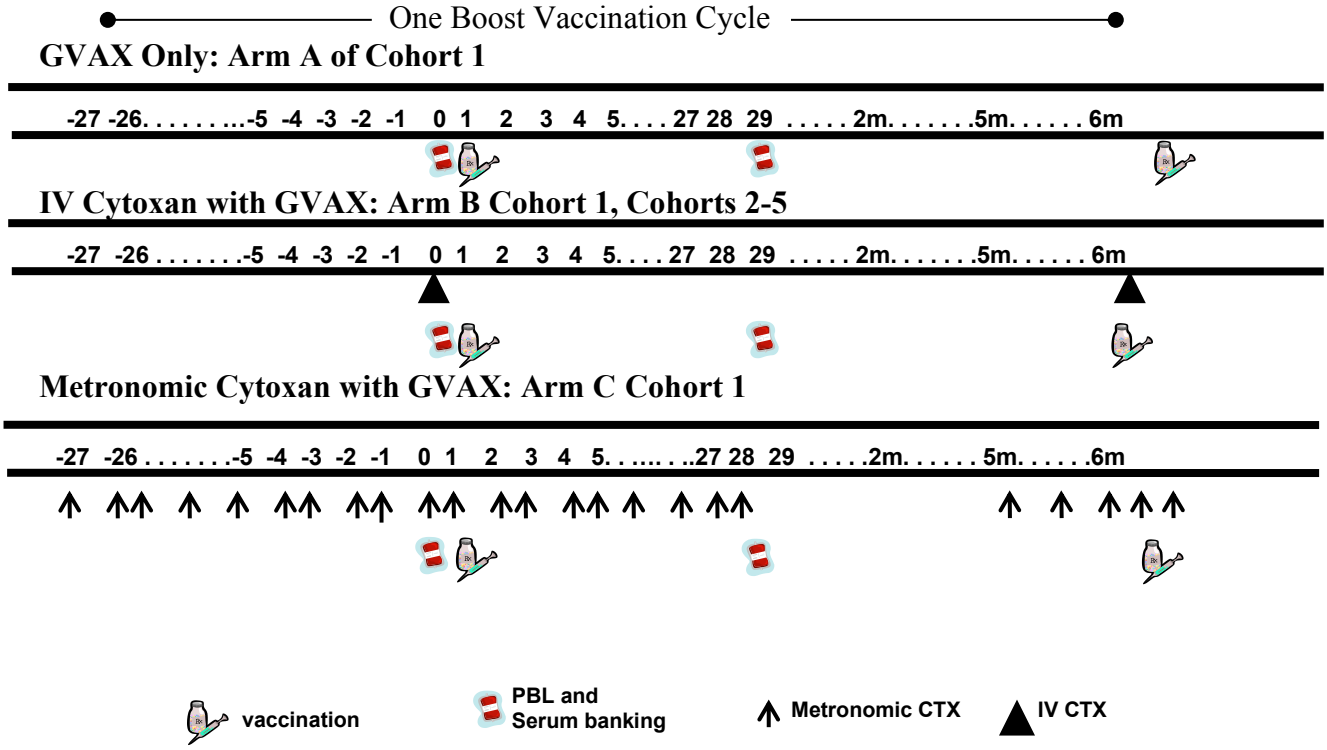


Figure 2 Schema for One Boost Vaccination Cycle



2.0 Background and Rationale

2.1 Introduction

Pancreatic cancer is the fourth leading cause of all cancer deaths. Although only 37,680 Americans are expected be diagnosed with pancreatic cancer, 34,290 will die from pancreatic cancer in 2008 (American Cancer Society, 2008). Only about 20% of patients diagnosed with pancreatic cancer will be eligible for surgical resection with a pancreaticoduodenectomy, the only potentially curative treatment. However, even among those patients who undergo surgery and adjuvant therapy 79% will eventually die of recurrent disease (Ahlgren, 1996). Pancreatic cancer has the most dismal prognosis among 18 cancer diagnoses. The statistics for 1996-2003 for the five-year relative survival rates for pancreatic cancer by stage are: 5.0% for all stages, 20.3% for localized, 8.0% for regional, and 1.7% for distant (American Cancer Society, 2008). Despite significant efforts to develop new therapies, locally advanced unresectable disease has a median survival of 10-12 months and subjects with metastatic pancreatic adenocarcinoma have a median survival of 3-6 months. While surgical resection is the only curative option, the majority of subjects (80-85%) present with advanced unresectable disease. These dismal survival rates require the development of novel approaches for the treatment of pancreatic cancer.

2.2 Rationale for cell-based immunotherapy of pancreatic adenocarcinoma

Adjuvant chemoradiation with a 5-Fluorouracil (5-FU) containing chemotherapy regime has been the standard for patients undergoing complete pancreatic cancer resection. However, even the most recent studies have demonstrated only modest improvements in disease-free survival (Smeenk et al., 2005). Immunotherapy is a potentially therapeutic approach to the treatment of pancreatic adenocarcinoma for several reasons. First, immunologic killing of tumor cells acts by a mechanism that is distinct from standard chemotherapy and radiation therapy and may represent a non-cross resistant treatment modality. Second, the immune system is capable of recognizing a diverse array of potential antigens while orchestrating selective and specific cytotoxic responses. This may be particularly important in the killing of a heterogeneous tumor population while avoiding normal tissue toxicity. Third, preclinical animal models using a vaccine approach for immunotherapy have been able to eliminate small burdens of established tumors, a situation that corresponds to the state of minimal residual disease commonly found after resection of human tumors (Burris et al., 1997; Heineman et al., 2003). Fourth, a completed Phase I trial evaluating an allogeneic, irradiated, granulocyte-macrophage colony stimulating factor (GM-CSF) secreting tumor vaccine in patients with adenocarcinoma of the pancreas demonstrated both clinical and immunologic responses (Jaffee et al., 2001). In addition, an analysis of 60 patients in a phase II study testing the safety and efficacy of the vaccine has shown an 86% one-year survival and a 61% two-year survival (Laheru et al., 2007). There is also a proportion of patients from both the phase I and the phase II studies who remain disease-free. These studies, together with the increasing evidence that human tumor-specific antigens can be recognized by the immune system suggests that specific immune responses can be generated against pancreatic adenocarcinoma if the immune system is sufficiently primed.

Analyses of pre-clinical and human pancreatic tumors have recently demonstrated that immune checkpoints such as T regulatory cells are infiltrating pancreatic pre-malignancies (PanIN) and early pancreatic tumors (Le, Jaffee, Laheru, et al., unpublished data). Therefore, it will be

important to better understand how to deliver a vaccine together with checkpoint inhibitors even to patients with potentially curable cancers. The current J0810 clinical study is designed to test the hypothesis that metronomic cyclophosphamide will be more effective than single dose cyclophosphamide at inhibiting T regulatory cells, and improve pancreatic cancer immune responses induced by this allogeneic paracrine cytokine tumor vaccine when compared with vaccine alone. Unlike the prior clinical trials testing our pancreatic cancer vaccine, in the J0810 study the first immunization of vaccine occurs in the neo-adjuvant setting, two weeks prior to the Whipple procedure. This will allow us to test whether cyclophosphamide is effective at inhibiting T regulatory cells within the tumor and whether the early inhibition of T regulatory cells allows for the induction of earlier and more potent anti-tumor immune responses.

2.3 Vaccine-based Strategies currently undergoing testing in subjects with pancreatic adenocarcinoma

There are a number of vaccine approaches that have been tested or are undergoing testing in pancreatic cancer subjects. Most of these approaches target one candidate tumor antigen. Such targets include: MUC-1, CEA, and mutated k-ras. The approaches used have included immunization with HLA class I and class II peptides, immunization with the whole protein, or delivery by antibody, heat shock protein or dendritic cells (Apostolopos & McKenzie, 1994; Kabayashi, Terao, & Kawashima, 1992; Brossart, Heinrich, & Stuhler, 1999; Apostolopous et al., 1997; Abrams et al., 1996). To date, these studies have demonstrated the induction of T cell responses. Significant clinical responses have not yet been observed. This may be due to the lack of potency of these approaches, to the existence of host mechanisms of immune tolerance, or both. More recent pre-clinical studies suggest that combined vaccine approaches integrating vaccine with immunomodulatory agents are significantly more effective than vaccines alone in models of tumor tolerance (Reviewed by Prendergast and Jaffee, 2007).

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

We have 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 hours 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 (APC) responsible for priming immune responses against infectious agents and tumor antigens. Autologous 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 (Berns, et al., 1995; Simons et al., 1999; Soiffer et al., 2003).

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 (Cox et al., 1994; Kawakami, Eliyahu, & Delago, 1994). 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 APCs as the critical cells required to present immunogen to T cells in the context of MHC (Dranoff et al., 1993; Jaffee et al, 2001; Thomas et al., 2004). 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 (Jaffee et al., 1998). In addition, these cell lines carry the same k-ras mutation as the original tumor specimen that 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 secrete GM-CSF at 80-90 ng/ 10^6 cells/ 24 hrs for at least 5 days in culture (Jaffee et al., 1998; Jaffee et al., 1993). 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 (Jaffee et al., 1998). 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 one phase I and three phase II studies in both the adjuvant and metastatic setting. The clinical lots for this long term boost study will be manufactured and released by the GMP Cell Processing and Gene Therapy Facility at Johns Hopkins. .

2.5 Rationale of using low dose cyclophosphamide as an immune modulator

2.5.1 Introduction: Immune tolerance and regulatory T cells.

Efficient immunization against cancer requires a vaccine capable of eliciting potent CD4 and CD8 T cell immune response. However, tumors have evolved several mechanisms to escape immune surveillance, including immune tolerance involving immunosuppressive T lymphocytes (Drake, Jaffee & Pardoll, 2006). Tumors have been shown to induce rapid expansion of $CD4^+CD25^+$ regulatory T cells (Treg) in human and mice, leading to delayed rejection of immunogenic tumors (Liyanage et al., 2002; Woo et al., 2001; Curiel et al., 2004). Conversely, elimination of these Tregs, which constitute 1-3% of the peripheral $CD4^+$ T cell pool in naïve mice, elicited potent antitumor immune responses leading to tumor eradication (Shimizu et al., 1999; Sutmuller et al., 2001).

Cyclophosphamide has been studied for its role in breaking the immune tolerance. For decades, it is known that low dose and high dose cyclophosphamide have different biological activities when it is used as a chemotherapy agent (Motoyoshi, et al., 2006). High dose cyclophosphamide has a cytotoxic activity; whereas, low dose cyclophosphamide has predominantly an immune modulating effect. Cyclophosphamide, as a cytotoxic chemotherapy agent, is used in treating multiple types of cancer including breast cancer, lymphoma, leukemia, sarcoma, etc. In addition, cyclophosphamide is also used as an immune suppressor in autoimmune diseases.

2.5.2 Evidence from animal studies supporting low dose cyclophosphamide as an immune modulator

Immune modulating doses of cyclophosphamide-containing chemotherapy have been studied in mouse tumor models in conjunct with vaccination. For example, Dr. Jaffee and colleagues have been studying the rodent *HER-2/neu* transgenic mouse model of mammary tumors (*neu*-N). These mice are a clinically relevant model of breast cancer. They develop spontaneous HER-2/neu expressing mammary tumors following over expression of the transgene under the murine mammary tumor virus (MMTV) promoter in normal mammary tissue. It has been demonstrated that vaccination of these mice induces weak HER-2/neu specific B cell and T cell responses that are not capable of controlling mammary tumor growth regardless of the vaccine approach. Dr. Jaffee's group (Ercolini et al., 2005) reported that treatment of these *neu*-N mice with such doses of cyclophosphamide before vaccination resulted in tumor rejection in 10-30% of mice, which were otherwise given vaccination alone and would all become immune tolerant to the tumor growth. Interestingly, it was also shown that this vaccine-enhancing effect of cyclophosphamide is mediated through selectively inhibiting the cycling population of $CD4^+CD25^+$ Treg cells and recruiting high-avidity antitumor effector T cells. 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.

2.5.3 Rationale for using daily metronomic cyclophosphamide for immune modulation

In previous clinical trials, a single intravenous dose of cyclophosphamide was given one day prior to each vaccination. Such a schedule was found to be effective in enhancing vaccine induced anti-tumor immune responses by inhibiting regulatory T cell activity in the *neu*-N mouse model of mammary tumors. In this animal study, a single dose of cyclophosphamide given by injection one day prior to vaccination, consistently led to tumor rejection response rates of no more than 30% of treated mice. Although these responses are statistically better than what is observed in mice treated with vaccine alone (0% cures), there is clearly room for improvement. Evaluation of the kinetics of Treg depletion following the one dose of cyclophosphamide revealed that about 50% of Tregs are depleted by 48 hours, but the population then rebounds reaching normal peripheral blood and lymph node levels by 7-14 days after treatment with cyclophosphamide. These findings raised the possibility that the success of cyclophosphamide modulation depends on the long-term depletion or inhibition of Treg cell function since the Treg cell population in *neu*-N mice recovers within 1-2 weeks of cyclophosphamide treatment. Repetitive intravenous cyclophosphamide at these doses is not a good option because of the concern that cyclophosphamide would induce lymphopenia and inhibit the cycling of antigen-specific effector cells after vaccination, thereby abrogating the vaccine-induced antigen-specific T cell response.

Recent studies have suggested that metronomic low-dose cyclophosphamide may provide more prolonged depletion or inhibition of Treg cell function and more effectively synergize with cancer vaccines in mouse models (Taieb et al., 2006; Hermans, et al., 2003; Ghiringhelli et al., 2004; Lutsiak, et al., 2005). Metronomic cyclophosphamide has also been tested for its role in anti-tumor angiogenesis in advanced human cancer patients (Orlando et al., 2006a; Orlando et al., 2006b; Glode et al., 2003; Bottini et al., 2006; Suvannasankha et al., 2007). Prolonged treatment with metronomic cyclophosphamide results in prolonged clinical benefit, and with minimal acute or delayed toxicity. Ghiringhelli et al. (2007) more recently demonstrated that metronomic cyclophosphamide as a single agent treatment selectively depletes $CD4^+CD25^+$ regulatory T cells and restores T and NK effector function in end stage cancer patients. This study provides the rationale for testing recurrent treatment with metronomic cyclophosphamide in sequence with our pancreatic cancer vaccine as a valuable combination for reducing tumor-induced immune tolerance and synergizing with the anti-tumor effect of the cancer vaccine.

The above studies provide rationales for the ongoing J0810 (neo)adjuvant pancreatic vaccine trial to employ the combinatorial treatment with vaccine and cyclophosphamide given at 50 mg twice daily for one week on followed by one week off. This metronomic cyclophosphamide schedule was demonstrated to be safe and effective in modulating immune suppressive activity by Ghiringhelli et al. (2007).

2.6 Phase I study of lethally irradiated allogeneic pancreatic tumor cells transfected with the GM-CSF gene. Phase I study at Johns Hopkins

2.6.1 Summary of Study Design

This study was the first clinical trial to test the hypothesis that allogeneic GM-CSF secreting pancreatic tumor cell lines can prime a systemic immune response in subjects with resected pancreatic adenocarcinoma. Fourteen subjects with stage 2 or 3 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.

2.6.2 Toxicity Events

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 (Davis, Dineen, Landa et al., 1999). The most frequently occurring toxicities of the vaccine were injection site reactions. See **Table 1**. for a summary of the toxicities. All were grade 1 or 2 by the National Cancer Institute (NCI) Common Toxicity Criteria. The total number of injection site reactions in the phase I allogeneic pancreatic tumor vaccine study was 28 out of 30 (93%) vaccine treatments. Patients who received the second, third, and fourth dose levels of the allogeneic pancreatic tumor vaccine all had grade 2 injection site reactions. Of these grade 2 injection site reactions all had erythema and induration,

with 69% (18/26) of the injection site reactions also having local pruritus at the vaccine sites. One patient experienced tenderness at the vaccine sites lasting up to three days after the first and third vaccine at dose level four. The patient required no analgesics. All injection site reactions were self-limiting and no one had any limitations on their activities of daily living related to these local signs and symptoms. Sixty-four percent (18/28) of the injection site reactions completely resolved within a week. Sixty-nine percent (9/13) of the patients who experienced injection site reactions were free of all local toxicities within a week. Thirty-one percent (4/13) of the patients experienced injection site reactions lasting more than a week with pruritus at the injection sites being the symptom of longest duration, lasting up to 41 days. Two of the four patients with the lengthy local toxicities also experienced systemic pruritus and rash. One of which was confirmed by biopsy to be Grover's Syndrome. The other person with the systemic rash and pruritus did not have a skin biopsy at that time. The same patient who experienced the Grover's Syndrome also developed recurrent swelling at the vaccine sites at that time which was seven to ten days after the second vaccine at level 4. After the third vaccine, this patient also had grade 1 lymphedema with erythema and swelling in the lymph drainage areas, particularly in the upper right extremity where two or the six vaccines were administered.

Systemic toxicities in these patients receiving Mitomycin-C and vaccine included: Grade 1 musculoskeletal stiffness and generalized pruritus lasting two hours. One patient on dose level 3 experienced acute anemia (hemoglobin dropped to 6.3 gm/dl), thrombocytopenia (platelets dropped to 5,800/mm3), asymptomatic jaundice (total bilirubin increased to 9.8 mg/dl, direct bilirubin to 7.8 gm/dl). This occurred approximately six weeks after completing Mitomycin-C containing chemotherapy. There was also asymptomatic anemia with hematocrit nadir of 19.8 %, hemoglobin nadir 6.6 g/dl and asymptomatic thrombocytopenia with platelet nadir of 77,000 / mm^3 during the chemotherapy course which included Fluorouracil (5-FU), Leucovorin, Mitomycin-C, and Dipyridamole (Persantine) prior to this event. These adverse events were consistent with Mitomycin-C associated thrombocytopenic purpura (TTP). Twenty-four days after the second vaccine at dose level three, and seven days after the blood transfusions, the same person experienced a grand mal seizure. It is unlikely that this adverse event is related to the vaccine. After receiving the vaccine this patient developed symptoms that have since been attributed to thrombotic thrombocytopenic purpura (TTP). Although we believe this condition was due to the Mitomycin-C treatment completed one month previously, this was reported to the IRB as a possible adverse event due to the temporal proximity to immunization with the vaccine. The patient was taken off study. The patient's condition improved, the symptoms of TTP resolved, and the patient remains in complete remission at this time. This adverse event has not recurred in the more than 200 additional patients who have been treated in follow-up vaccine studies with the same vaccine.

With dose level four, 50% (3/6) patients experienced a systemic reaction. One patient experienced grade 1 constitutional symptom of fatigue and grade 1 musculoskeletal, and achy joints. Another patient on vaccine dose level 4 experienced multiple vaccine related symptomatic toxicities, including: a grade 2 rash, systemic pruritus, and Grover's Syndrome, a grade 1 urticaria, and a grade 1 recall induration at the vaccine sites. In conclusion, the main toxicity clearly attributable to the vaccine is Grade 1 or Grade 2 and the vaccine seems to be well tolerated by patients undergoing their cancer treatment.

Table 1. Toxicity Events Associated with Phase I Allogeneic Pancreatic Tumor Vaccine

Toxicity*	Grade 1†		Grade 2		Grade 3		Grade 4	
	No. of Vaccine Events	No. of Patients Who Had Toxicity	No. of Vaccine Events	No. of Patients Who Had Toxicity	No. of Vaccine Events	No. of Patients Who Had Toxicity	No. of Vaccine Events	No. of Patients Who Had Toxicity
Local								
Erythema at vaccine site	1[a]	1	26[a,b,c,d]	12				
Induration at vaccine site			26[a,b,c,d]	12				
Pruritus at vaccine site	1[b]	1	18[a,b,c,d]	11				
Tenderness at vaccine site			2[d]	1				
Recall induration at vaccine site	1[d]	1						
Lymphedema of one extremity	1[d]	1						
Systemic								
Pruritus, not at vaccine site			3[b,d]	3				
Urticaria			1[d]	1				
Skin rash			2[d,e]	2				
Joint stiffness/pain	2[b,d]	2						
Fatigue	1[d]	1						
Other[f]					1[c,f]	1	1[c,f]	1

*Toxicities were graded using the National Cancer Institute's cancer clinical trials common toxicity criteria.

†The data represent the toxicities observed in the 14 patients who were treated with the vaccine. There were a total of 30 assessable vaccine treatments. Footnotes in table are defined as follows: a, occurred after dose level 1 (1×10^7 cells); b, occurred after dose level 2 (5×10^7 cells); c, occurred after dose level 3 (1×10^8 cells); d, occurred after dose level 4 (5×10^8 cells); e, one rash was biopsied to reveal Grover's syndrome; f, after receiving dose level 3 (1×10^8 cells), one patient experienced grade 2 thrombocytopenia, grade 2 elevated AST, grade 3 seizure, grade 3 anemia, grade 3 elevated ALT, and grade 3 elevated bilirubin. The symptoms were attributed to thrombotic thrombocytopenia purpura.

2.6.3 Disease Free Survival

There are three patients who participated in the original study who remain pancreatic cancer disease free. The pancreatic cancer disease free survival of patients is defined as the time interval from date of diagnosis with adenocarcinoma of the pancreas to the date of radiographic evidence of disease recurrence. Only 14 of the 15 patients who participated in this trial were considered for the disease free analysis, as one patient had stage 4 disease with liver metastasis prior to entering the study. An increase in the disease-free survival was associated with increasing total vaccine dose, which is equal to the dose level of cells multiplied by the number of doses received. Using the nonparametric correlation of Spearman's rho the association of total pancreatic tumor vaccine dose and disease free survival is statistically significant (p=0.028). Study participants had a 43% (6/14) one year disease free survival and 86% (12/14) one year overall survival. Of the fourteen patients with resectable disease in this study, three (21%) are still free of pancreatic cancer going on 10 years from the date of diagnosis of pancreatic cancer.

2.6.4 Immunologic Data

2.6.4.1 Serum GM-CSF levels

Systemic GM-CSF levels were evaluated as an indirect measure of the longevity of vaccine cells at the immunizing site. Serum GM-CSF levels were measured in the patients participating in the first pancreatic tumor vaccine study at time 0, 24, 48, 72, 96 and 120 hours following the first vaccine. As was observed in pre-clinical studies, GM-CSF levels peaked at 48 hours following vaccination. The peak concentration of serum GM-CSF levels was seen at 48 hours in 83% (5/6) of the patients who achieved a measurable serum GM-CSF level. Only one patient who received dose level one had a measurable serum GM-CSF level at a minimum level of 1.0 pg/ml at 48 hours. There was no measurable serum GM-CSF level in any of the six patients who received dose levels two and three at any time point. All five patients who received dose level four had measurable

serum GM-CSF levels with the range of 1.2 to 14.0 pg/ml. In addition, serum GM- CSF levels could be detected for up to 96 hours following vaccination. GM-CSF levels however became undetectable by 120 hours in all patients on study. No side effects other than asymptomatic eosinophilia were associated with these low but detectable serum GM-CSF levels. 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.

2.6.4.2 Local Immune Reaction of Vaccine Site

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.

2.6.4.3 Post-Vaccination DTH

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 this phase I pancreatic cancer vaccine trial, post vaccination DTH responses to autologous tumor cells were observed in 1 of 3 subjects receiving 1 X 10^8 and in 2 of 5 subjects receiving 5 X 10^8 vaccine cells (Jaffee et al., 2001). They are the three long-term survivors of the initial phase I study and are currently participants in the long-term follow-up study (SKCCC J0248/IRB # NA_00036444 [formerly 02-10-14-03]), remain disease-free and 2 subjects are receiving additional vaccinations. No additional long-term toxicities have been uncovered in this cohort.

2.6.4.4 Mesothelin-Specific T Cell Response

A correlation between the induction of post-vaccination mesothelin-specific T cell responses and disease-free survival in these three patients was observed (Thomas et al., 2004). Consistent with their long-term survival and post vaccination DTH responses, CD8+ T cell responses to multiple HLA-A2, A3, and A24-restricted mesothelin epitopes were observed exclusively in these three patients. Importantly, neither of the vaccinating pancreatic cancer cell lines expressed HLA-A2, A3, or A24, therefore providing the first direct evidence that CD8 T cell responses can be generated via cross-presentation by an immunotherapy approach designed to recruit APCs to the vaccination site. This result also suggests that the mesothelin-specific T cell response may serve as a better surrogate marker for the assessment of vaccine-induced immune response in the future vaccine studies. Thus, in the subsequent phase II study, the role of mesothelin-specific T cell response is a primary biomarker of immune response to further validate this marker as a correlate of vaccine activity (See Section 2.7 for detail).

2.7 Phase II study of lethally irradiated allogeneic pancreatic tumor cells transfected with the GM-CSF gene

2.7.1 Summary

A follow-up Phase II clinical trial of the pancreatic cancer vaccine in patients who received the vaccine after surgical resection of tumor and adjuvant radiation chemotherapy was conducted at

Johns Hopkins. All 60 planned subjects on the study completed the treatment phase in June 2006. The toxicities associated with the vaccine in this study include: local vaccine site skin reactions and systemic rashes similar in severity (grade 1-2) to what was observed in the phase I trial. There were no related serious adverse events observed. The one-year survival was 86% and the two-year survival was 61%. The last vaccination received by patients completing the study was at 18 months. The median overall survival was 24.8 months, which compares favorably with a previously reported median of 21 months. A correlation between disease-free survival and the induction of mesothelin-specific T cell responses was observed.

2.7.2 Study Design

Sixty research participants received at least one and a maximum of five vaccinations of two pancreatic cancer cell lines each delivering 2.5 x 10^8 cells intradermally distributed among three lymph node regions following enrollment into the Johns Hopkins study, IRB # 00-01-13-02 (SKCCC J9988) titled, A safety and efficacy trial of lethally irradiated allogeneic pancreatic tumor cells transfected with the GM-CSF gene in combination with adjuvant chemoradiotherapy for the treatment of adenocarcinoma of the pancreas. See **Table 2** for the demographic details of this study. Enrollment was completed in January of 2005. Vaccine one was administered 8-10 weeks following surgical resection. Patients were subsequently treated with 5-fluoruracil (5-FU) continuous infusion based chemotherapy integrated with radiotherapy. Research participants who remained disease-free one month after completing the chemoradiotherapy received the vaccines two through four one month apart. A fifth and final vaccine was administered six months after the fourth vaccine to those subjects who remained disease-free.

Table 2. Research Participants' Demographic Data Characteristic (N=60)

Male	37
Female	23
Median age	56.7
Age range	41-83
Node-positive	52
Margin-positive	18
Node- and Margin-positive	18

2.7.3 Safety Data

Based on an early analysis, we conclude that the administration of the GM-CSF allogeneic cancer vaccine is safe and well tolerated. Treatment related side effects were similar to those side effects seen in the phase I study. The most common side effects were vaccine injection site reactions of induration and erythema that were transient in all research participants. In addition, some subjects also had transient vaccine injection site reactions of tenderness and pruritus. The systemic reactions included transient elevation in eosinophil counts, rashes and flu-like symptoms that have included low grade fever, chills, malaise, arthralgias, myalgias, and fatigue. Most patients had a transient elevation in their eosinophil count which demonstrates the bioactivitiy of GM-CSF. All vaccine related toxicities have been of the same intensity and duration as those observed in the phase I study (Jaffee et al., 2001).

2.7.4 Survival data

With a median follow up of 24.7 months (Lutz et al., manuscript submitted), median disease-free survival is 17.3 months (95% CI: 14.6 – 22.8) with median overall survival of 24.8 months (95% CI: 21.2 - 31.6). While this study was not designed to be directly compared to our surgery database, a planned cohort analysis of patients resected at Johns Hopkins who received chemoradiation without immunotherapy demonstrated a median overall survival of 20.3 (95% CI: 18.0 – 23.9) months. The percentage of lymph node-positive patients (most important predictor of early recurrence) in this phase II study, 88%, was higher than those patients enrolled in other published PDA adjuvant studies (50-71%). In addition, 10 patients had a post-resection CA19-9 > 90 U/ml; whereas similar patients would have been excluded from a recently completed phase III (CONKO-1) study comparing 6 months of adjuvant gemcitabine to surgery alone. Nevertheless, the median overall survival of 24.8 months compares favorably to two recently published adjuvant studies (22 months in the CONKO-1 study and 20.6 months in the RTOG 9704 study)[2]. These encouraging preliminary clinical data in 60 patients at Johns Hopkins provided the impetus for the NCI Gastrointestinal Cancers cooperative group, CALGB, to conduct a follow up multicenter study. The multi-center study of the pancreatic vaccine in the adjuvant setting is under development and should begin enrolling by the end of 2009.

2.7.5 Immunologic Data

Peripheral blood lymphocytes (PBL) from two patients demonstrating prolonged disease-free survival 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 an 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. In total, 8 HLA-A0101-binding and 6 HLA-A0201-binding mesothelin-derived T cell epitopes have been identified. IFNγ ELISPOT analyses were performed to measure the frequencies of CD8$^+$ T cells specific for these peptides prior to and following each vaccination in the 25 HLA-A0101$^+$ and 23 HLA-A0201$^+$ patients, and to 1 HLA-A0207$^+$ patient covering 43 of the 60 patients (6 patients expressed both HLA-A1 and HLA-A2). These studies showed that CD8$^+$ T cells specific for at least one mesothelin peptide were detected following the first vaccine treatment in 38 of the 43 subjects analyzed (Lutz et al. Manuscript submitted). Four of the 5 patients for whom mesothelin peptide-specific T cells were not detected following the first treatment recurred before the second scheduled immunotherapy treatment. In addition, when compared to pre-treatment levels, enhanced mesothelin-specific T cell responses following the first vaccination were only observed in patients who went on to receive multiple treatments. Furthermore, within the group of patients who received multiple vaccinations, the maintenance of enhanced mesothelin peptide-specific responses throughout the course of treatment was associated with improved disease-free survival (**Figure 3**). We also measured responses to negative control peptides (tyrosinase peptides) and a positive control CMV/EBV/Influenza A (CEF) peptide pool. Negative control responses were low or undetectable whereas CEF pool responses were detected in all subjects analyzed, but post-vaccination changes in CEF pool-specific responses were not detected in any of the groups. Combined, these data suggest that the changes measured were not due to non-specific time point-related differences but

reflect actual changes in mesothelin-specific CD8$^+$ T cell responses. Importantly, these studies demonstrated that different patients responded to different epitopes and rarely to the complete panel of peptides following the first vaccination. Surprisingly, enhanced T cell responses were observed to an average of approximately 50% of mesothelin peptides following the first vaccination in both patients remaining disease-free and patients with progressing disease. However, an expansion in the mesothelin epitope-targeted repertoire following boost vaccinations was observed almost exclusively in patients who demonstrated longer disease-free survival (**Figure 4**).

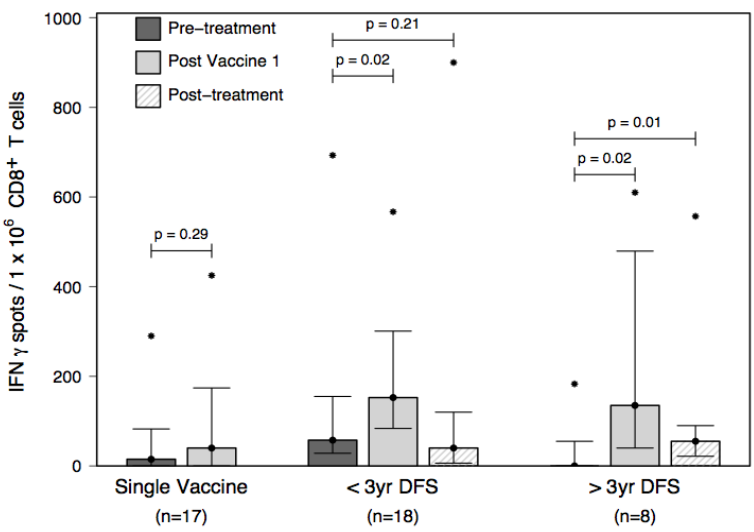


Figure 3. Post-immunotherapy enhancement of mesothelin-specific CD8$^+$ T cell responses correlates with disease-free survival. IFNγ ELISPOT assays were performed to measure the frequencies of CD8$^+$ T cells specific for mesothelin epitopes in PBL isolated from HLA-A1$^+$ and HLA-A2$^+$ patients for which pre and post treatment lymphocytes were available. Patients were divided into three groups: those receiving only one treatment (Single Vaccine, n=17), those receiving multiple treatments who recurred within 3 years (DFS < 3yr, n= 18), and those receiving multiple treatments who remained disease-free for greater than 3 years (DFS > 3yrs, n=8). Mesothelin responses are reported for the epitope to which each individual patient showed a maximum response. Shown for each group is the median number of IFNγ-secreting CD8$^+$ T cells per 1x10^6 CD8$^+$ T cells above background measured against irrelevant tumor antigens with the inter-quartile range and the maximum value (*). Responses were measured prior to treatment (Pre-treatment), 14 days following the first immunotherapy treatment (Post Vaccine 1) and if given multiple treatments, 28 days following the final immunotherapy treatment (Post-treatment). Post-immunotherapy responses were compared to pre-treatment responses using two-tailed Wilcoxon sign-rank tests and the calculated p-values are shown.

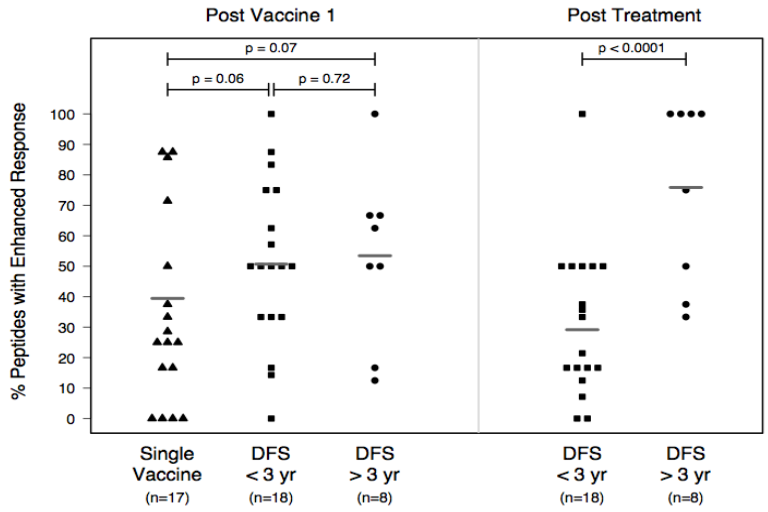
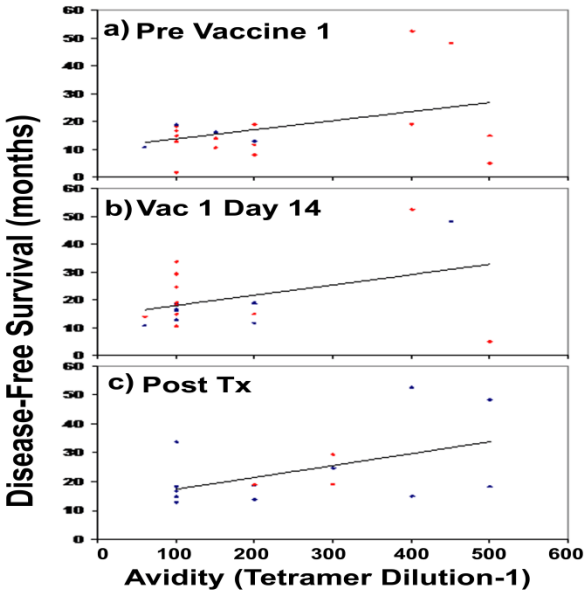


Figure 4. Longer disease-free survival is associated with an expansion in the CD8$^+$ T cell repertoire targeting mesothelin epitopes. IFNγ ELISPOT assays were performed to measure the frequencies of CD8$^+$ T cells specific for mesothelin epitopes as described in **Figure 1**. Shown are the percentage of mesothelin peptides per patient for which a post-immunotherapy enhancement, defined as a 2-fold or greater increase in the number of IFNγ-producing peptide-specific T cells, was measured following the first (Post Vaccine 1) and the final immunotherapy treatments (Post treatment, only for patients receiving multiple treatments). Bars represent the overall percentage of peptides for which an enhanced response was measured for each group. Group repertoires were compared using logistic regression and the calculated p values are shown.

Dilutional tetramer analysis was also performed on PBL isolated prior to 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 disease-free survival. **Figure 5** shows the relationship between the avidity of $MesoA2_{(530-538)}$-specific T cells and disease-free survival. For each of the 6 mesothelin peptides evaluated, a trend toward improved disease-free survival 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 would suggest that higher avidity pre-committed T cells are sometimes already present in resected pancreatic cancer patients that can be activated with vaccination. 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 6** 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.

Figure 5. Meso $A2_{(530-538)}$-specific T cell Avidity vs Disease-Free Survival. 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 disease-free survival. Linear regressions were performed and the trendlines 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 γM tetramer stock solutions.



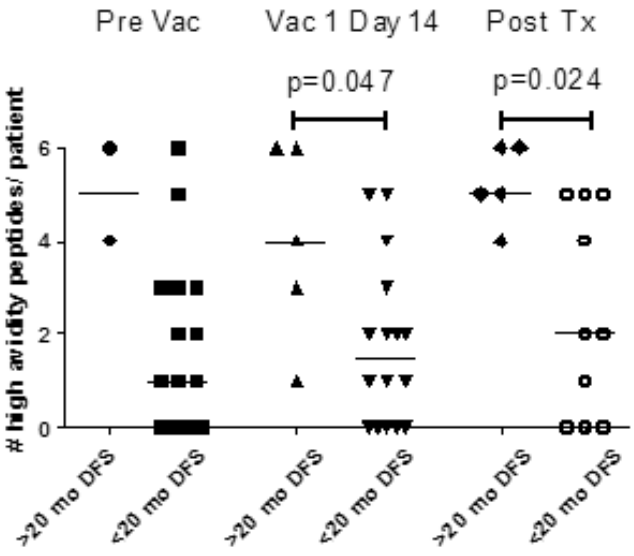


Figure 6. Longer Disease-Free Survival is Associated with Larger High Avidity Mesothelin-Specific CD8+ T Cell Repertoires. Dilutional tetramer analysis was performed for each of the 6 HLA-A2-binding mesothelin epitopes on PBL isolated prior to vaccination (Pre Vac), 14 days following the 1st vaccination (Vac 1 Day 14) or 28 days following the final vaccination (Post Tx) from HLA-A2+ patients. Dilutions were performed using 1 □M tetramer stock solutions. Patients were divided into 2 groups: those who recurred within 20 months (< 20 mo DFS) and those who remained disease-free for greater than 20 months (> 20 mo DFS). 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. When group sizes were large enough, high avidity repertoires were compared between groups using two-tailed Wilcoxon sign-rank tests and the calculated p-values are shown.

2.8 Phase II Study of the GM-CSF allogeneic vaccine alone and given in sequence with immune modulating doses of Cyclophosphamide in subjects with advanced pancreatic cancer

2.8.1 Summary

As briefly cited above, we have completed a feasibility study of our GM-CSF allogeneic vaccine administered alone or in sequence with Cyclophosphamide in subjects with advanced pancreatic cancer (Laheru et al., 2008). This study was an open label multi-center study sponsored by Cell Genesys, Inc in collaboration with US Oncology. Subjects were enrolled into one of two cohorts: Cohort A- 30 subjects administered a maximum of six doses of the same pancreatic cancer vaccine as described above using the two pancreas cancer cell lines each delivering 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 the safety and induction of immune responses when treated with either vaccine alone or in sequence with cyclophosphamide. Secondary objectives include time to disease progression (TTP) and median overall survival (OS).

2.8.2 Safety and Efficacy Data

We have reported the following findings (Laheru et al., 2008):

1. The administration of a GM-CSF allogeneic pancreatic cancer vaccine is safe both alone and when given in sequence with cyclophosphamide. It is well-tolerated by patients with advanced pancreatic cancer, and the majority of these patients had received two or more 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.

2. 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.

3. Stable disease was noted in 16.7 % of subjects in Cohort A (vaccination alone) and 40% of subjects in Cohort B (vaccination plus cytoxan). 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 chemotherapy in 12/20 subjects for Cohort B and in 30/50 subjects overall.

2.8.3 Immunologic Data

Immune analyses in a subset of patients with available lymphocytes isolated at a number of time points following multiple immunizations demonstrated a correlation of increased mesothelin-specific $CD8^+$ T cell responses with progression-free survival (**Table 3**). $CD8^+$ T cell response to HLA restricted mesothelin epitopes were augmented post cycle 3 and 6 of the therapy predominantly in patients treated with Cytoxan + vaccination (9 of 10) as compared with patients treated with vaccination alone (4 of 8). $CD8^+$ T cell responses were detected against the positive control CMV/EBV/Influenza A (CEF peptide) pool in all patients treated in both Cohorts. While the analysis to date is limited to HLA- $A1^+$, $-A2^+$ and $-A3^+$ patients, median survival from this subset of patients with induction or enhancement of mesothelin-specific T cell responses treated with immunotherapy alone is 7.6 months versus 10.4 months for patients treated with cyclophosphamide + immunotherapy.

Table 3. Summary of mesothelin-specific T cell responses in the HLA-$A1^+$/ HLA-$A2^+$/ HLA-$A3^+$ patients treated with the vaccine alone or in sequence with Cytoxan

Patient/HLA	Mesothelin-specific T cells/10^6 CD8 T cells						Survival (mo)
	Pre	Vaccine 3	Vaccine 6	Follow-up	Follow-up 2	Follow-up 3	
Cohort A (patients given immunotherapy alone)							
1/(2)	40	110	NA	NA	NA	NA	3.36
2/(1)	10	0	0	NA	NA	NA	7.1
3/(3)	20	10	NA	NA	NA	NA	3.36
4/(3)	20	50	100	30	50	NA	7.9
5/(1)	90	160	110	NA	NA	NA	17.6
6/(2)	40	60	NA	NA	NA	NA	6.1
7/(2)	60	0	NA	NA	NA	NA	1.7
8/(2)	0	0	NA	NA	NA	NA	2.86
Cohort B (patients given immunotherapy + Cytoxan)							
1/(2)	50	230	NA	NA	NA	NA	3.23
2/(1)	10	60	NA	NA	NA	NA	22.5
3/(2)	110	270	NA	NA	NA	NA	6
4/(2)	240	400	NA	NA	NA	NA	7.73
5/(3)	0	0	210	70	NA	NA	25+
6/(3)	70	130	190	NA	NA	NA	8.13
7/(2)	0	0	10	10	NA	NA	13.07
8/(2)	130	350	50	NA	NA	NA	3.7
9/(2)	30	60	50	30	40	110	12.3
10/(2)	50	100	NA	NA	NA	NA	2.6

NOTE: The ELISPOT assay was used to determine the number of mesothelin-specific $CD8^+$ T cells specific for the HLA-$A1^+$ epitope, $MesoA1_{(20-28)}$, HLA-$A2^+$ mesothelin epitope $MesoA2_{(530-539)}$, or the HLA-A3 $mesothelin_{(225-234)}$ epitope. All time points for each patient were assayed simultaneously in six replicates and reported as the mean number of mesothelin-specific $CD8^+$ T cells per 10^6 total $CD8^+$ T cells. Background spots were determined using a negative peptide control known to bind to HLA-A1 HIV-$Nef_{(73-87)}$ (QVPLRPMTY), HLA-A2 HIV-$gag_{(77-85)}$ (SLYNTVATL), and HLA-A3 HIV-1 $NEF_{(94-102)}$ (QVPLRPMTYK6). Background spots ranged from 0 to 10 spots per well. A CEF pool was used as a positive control. The CEF pool contains epitopes from cytomegalovirus, EBV, and influenza A virus proteins that bind to most HLA class I molecules. The CEF pool was obtained from NIH AIDS Research and Reference Reagent Program, Division of AIDS, National Institute of Allergy and Infectious Diseases, NIH (CEF control peptide pool, 9808). Positive control spots ranged from 40 to 1,300 per well.
Abbreviations: Pre, pretreatment 1; treatment cycle 1, posttreatment 1; vaccine 3, posttreatment 3; vaccine 6, posttreatment 6; NA, not available due to patient progression.

In addition, there was a trend toward prolonged progression-free survival in those patients who demonstrated persistent mesothelin-specific T cell responses with therapy. In this same study, mesothelin-specific T cells from a subset of HLA-$A2^+$ subjects were assessed for avidity by dilutional HLA-A2/$Meso_{(20-28)}$ and HLA-A2/$Meso_{(530-538)}$ tetramer analysis and the data are summarized in **Table 4**. As an example, tetramer analysis of cohort B subject 7 showed an increase in the frequency and avidity of mesothelin-specific T cells in post-treatment PBL compared to PBL isolated prior to treatment. In contrast, a decrease in post-treatment mesothelin-specific T cell frequency and avidity was measured in cohort B subject 8. Whereas changes in frequencies of mesothelin-specific T cells were detected, changes in frequencies of T cells specific for tyrosinase, an irrelevant melanoma antigen, were not detected in any of the 9 subjects evaluated (Laheru et al. 2008). This suggests that the changes measured were not due to time point-related differences in non-specific tetramer staining. Although the analysis was performed on a small number of subjects, it is interesting that the post-treatment $MesoA2_{(530-538)}$ tetramer titration was associated with overall survival. In the same cohort B, subject 7 had a survival of 13.07 months whereas subject 8 only had a survival of 3.7 months (**Table 4**). Importantly, these tetramer changes appear to be antigen specific since time point differences in tyrosinase tetramer titrations were not observed.

Table 4. Summary of HLA-A2 tetramer titrations in a subset of HLA-A2+ patients treated with the vaccine alone or in sequence with cyclophosphamide.

Patient	$MesoA2_{(531-539)}$		Tyrosinase		Survival (mo)
	Pre	Vaccine 3	Pre	Vaccine 3	
Tetramer titration (patients without cytoxan)					
4	1:60	1:60	1:10	1:10	7.9
6	1:40	>1:60	1:20	1:20	6.1
7	1:20	1:40	1:10	1:20	1.7
Tetramer titration (patients given cytoxan)					
1	1:10	1:40	1:10	1:10	3.23
4	1:20	>1:40	1:20	1:20	7.73
7	1:40	1:60	1:20	1:20	13.07
8	>1:60	1:20	1:10	1:10	3.7
9	>1:60	>1:60	1:20	1:20	12.3
10	1:60	<1:60	1:10	1:10	2.6

NOTE: Patient PBL were labeled with HLA-A2 tetramers as described in Patients and Methods. The tetramer dilutions at which detectable tetramer staining was lost are shown.
Abbreviations: Pre, prevaccine 1; vaccine 3, prevaccine 3.

2.9 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

At present, the JHMI IRB approved J0810 study titled, 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, 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 a 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 will be 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 10 patients (between 7/30/08 and 12/31/08). All ten patients enrolled into the study have received their first (neoadjuvant) cycle of treatment and underwent tumor resection (**Table 5**). Because a biopsy-proved diagnosis is not routinely obtained as part of standard of care prior to the surgery, this study does not require a biopsy for the study entry. With the improvement of pre-operative imaging techniques, the misdiagnosis of PDA is uncommon. Nonetheless, the participants were informed that there is a small chance of having a tumor different from PDA. Only one patient turned out to have ampullary adenocarcinoma and was subsequently removed from the study. Ampullary cancers are not eligible for this vaccine study because they have a different biology and likely different tumor antigens than those expressed by our vaccines which derive from PDA of the pancreatic head and neck. Another three patients did not meet eligibility criteria for the study continuation due to disease recurrence [redacted] and unresolved post-operative complications at the time of screen visits prior to the second vaccination cycle [redacted] Such a drop-off rate is anticipated. Therefore, six patients remain in the study, and three of them have gone far enough to receive the second vaccination. We estimate that this study needs to recruit an additional 50-54 patients for the accrual of 39 evaluable patients during the grant award period.

[redacted]

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 (**Table 5**) 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. 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. In addition, these preliminary data demonstrate the feasibility of enrolling and completing this study in an acceptable time frame.

Building on our prior experience in clinical development of our pancreatic cancer vaccine, we anticipate that the J0810 study will demonstrate the safety and feasibility of giving the vaccine in the neoadjuvant setting and in combination with immune modulating doses of cyclophosphamide. We also anticipate that vaccine-induced immune response is associated with an improved survival of patients. We are designing this trial as the follow-up study of the same subjects in the J0810 study and designed to test the hypothesis that boosting with the combinatorial treatment of vaccine and immune modulating doses of cyclophosphamide results in durable suppression of Tregs and consequently induces more durable immune responses that are associated with a continuous pancreatic cancer free survival.

2.10 Rationale of boost vaccinations and the long term treatment of immune modulating doses of cyclophosphamide

Published studies and our immune analysis with previous vaccine studies suggest that antigen-specific T cell response is often not sustained after the last prime vaccination and that diminishing T cell response may be associated with early disease recurrence. We have recently completed the accrual of the J0619 study which is testing the safety and efficacy of boosting the patients who remain disease free in our phase I and phase II vaccine studies and who have received four vaccinations in the J0619 study. The preliminary safety analysis suggests that boost vaccinations are safe with low grade local and systemic toxicities that have been observed with prime vaccinations. The preliminary immune analysis also suggests that sustained immune response is observed in patients who are receiving boost vaccines.

A more intriguing question is whether sustained Treg suppression is required for sustained T cell response. It is believed that suppression on Treg will diminish after metronomic cyclophosphamide is stopped. Our preclinical model showed that Tregs start to bounce back in one week following the treatment of one single intravenous low-dose cyclophosphamide (Ercolini et al., 2005); and in patients, peripheral Tregs started to

bounce back in two months after the completion of daily oral metronomic cyclophosphamide (personal communication). It thus will be important to explore whether the avidity and quantity of mesothelin T cell response start to drop after the treatment of vaccine and cyclophosphamide is completed through the prime vaccination protocol (J0810). Additionally, we wish to discern whether long-term intermittently administrated low-dose cyclophosphamide can reactivate T cells and whether low dose cyclophosphamide can further induce T cell response in combination with boost vaccinations.

Long term treatment with metronomic cyclophosphamide has been widely tested in clinical trials for a variety of metastatic cancer diseases and demonstrated to be safe. It has been tested as a daily maintenance therapy in combination with gleevec in treating patients with Gastrointestinal stromal tumor (GISTs) and also demonstrated to be safe and well tolerated (Personal communication). Long-term metronomic cyclophosphamide is also used in the rheumatology clinic for patient with certain conditions of vasculitis.

The suppressive effect of one-month metronomic cyclophosphamide on Tregs was reported. It is however not known what is the optimal duration of metronomic cyclophosphamide treatment for Treg suppression prior to each vaccination. In this study, for Arm C participants, metronomic cyclophosphamide will be given for one month to achieve Treg suppression prior to each boost vaccination. Thus, metronomic cyclophosphamide will be continued for one more month post vaccination.

In this boost vaccine study, metronomic cyclophosphamide will be given to Arm C participants at 50 mg daily, instead of 50 mg twice daily one week on and one week off. The total monthly accumulated dose of cyclophosphamide remains to be same and is thought to be equivalent in immune modulation. The 50 mg daily regimen is recommended in the design of immunotherapy studies of other malignant diseases (personal communication). Such a regimen is also the one that was most frequently used in published non-immunotherapy cancer treatment studies. Importantly, its convenience will make participants more compliant with the long-term treatment.

2.11 Rationale for Immunobiologic Endpoints

The major limitation to developing cancer vaccines has been the lack of identified pancreatic tumor antigens that are the known targets of the immune response. As such, current immune based approaches either target a small group of candidate antigens expressed by the tumor or rely on whole tumor cells as the immunogen. However, with the recent sequencing of the human genome and the development of rapid methods for identifying genes that are differentially expressed by tumor cells (Iacobuzio-Donahue, Maitra, Shen-Ong et al., 2002), potential candidate immune targets are being discovered that may serve as immunogens for treatment as well as prevention.

Mesothelin, a transmembrane glycoprotein member of the mesothelin/megakaryocyte potentiating factor (MPF) family was identified by differential gene expression to be over expressed by most pancreatic adenocarcinoma (Argani, Iacobuzio-Donahue, Ryu et al.,

2001). In the phase I GM-CSF pancreatic cancer vaccine study, we observed a post-vaccination induction of mesothelin-specific T cell responses in uncultured $CD8^+$ T cells isolated from the three subjects who were long-term survivors after vaccination. However, the patients who relapsed, we did not observe evidence of mesothelin recognition in post-treatment lymphocytes (Thomas et al., 2004). These data suggest that mesothelin may serve as a biomarker of vaccine-induced T cell responses. Finally, follow up data from our recently completed phase II adjuvant clinical trial provides further support that the post-vaccination induction of mesothelin specific T cell responses is associated with improved disease-free and overall survival in treated patients (see section 2.7.5)

Therefore, in this study, we will further evaluate the mesothelin specific T cell response as a biomarker of vaccine induced immune response. Specifically, we will compare the effects of vaccine boosts alone versus vaccine boosts given with either a single dose of intravenous cyclophosphamide or daily metronomic cyclophosphamide on changes in the number and function of peripheral mesothelin-specific $CD8^+$ T cells. Three parameters of vaccine induced mesothelin-specific T cell function will be assessed in this study as well as their association with disease-free and overall survival: 1) T cell number and cytokine expression; 2) T cell avidity; and 3) T cell repertoire changes. For exploratory purpose, we will assess whether sustained T cell function, measured by the repertoire and avidity of mesothelin specific CD8+ T cells, is associated with long-term combinatorial treatment with immune modulating doses of cyclophosphamide, and whether it is associated with prolonged survival.

3.0 Study Design and Treatment Plan

3.1 Study Overview

This vaccine boost trial will evaluate an equal mixture of two allogeneic GM-CSF secreting pancreatic vaccine cell lines, Panc 10.05 and Panc 6.03, in addition to low dose cyclophosphamide either as a single intravenous dose or as multiple metronomic oral doses for: (1) safety of administration, (2) disease-free and overall survival in patients with resected adenocarcinoma of the head, neck, or uncinate of the pancreas, (3) induction of mesothelin-specific T cell immune responses, and (4) number and function of Treg cells systemically.

Candidates for this study will include the following patient Cohorts who have a history of surgically resected pathologic stage 1 (no direct tumor extension beyond pancreas and no regional lymph node metastases), 2a (direct extension of tumor beyond pancreas), and/or 2b (regional lymph node metastases) adenocarcinoma of the head, neck, tail, or uncinate of the pancreas:

- **Cohort 1**: Patients who have previously participated in the study titled "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" [J0810, NA_00015858 (formerly 00-01-58-58)],

have no radiographic evidence of pancreatic disease recurrence, and have received the GM-CSF secreting allogeneic pancreatic cancer vaccine.

- **Cohort 2**: Patients who never received any type of pancreatic vaccine or immunotherapy, had the Whipple surgery within 18 months and completed the planned adjuvant chemotherapy and/or chemoradiation. Have no radiographic evidence of pancreatic cancer disease recurrence. Have not received any anti-cancer therapy in the past 28 days.
- **Cohort 3**: Patients who have previously participated in the study titled "A Randomized Study of a GM-CSF secreting allogeneic pancreatic cancer vaccine with or without a PD-1 Blockade Antibody (Nivolumab) for the Neoadjuvant and Adjuvant Treatment of Patients with Surgically Resectable Adenocarcinoma of the Pancreas" (IRB00050517, J1568), have no radiographic evidence of pancreatic disease recurrence, and received their sixth vaccine within 12 months of enrolling in J09100.
- **Cohort 4**: Patients who have previously participated in the study titled "A Phase II Study of GM-CSF secreting allogeneic pancreatic cancer vaccine in combination with PD-1 Blockade Antibody (Pembrolizumab) and Stereotactic Body Radiation Therapy (SBRT) for the Treatment of Patients with Locally Advanced Adenocarcinoma of the Pancreas" (IRB00083132, J15237), have no radiographic evidence of pancreatic disease recurrence, and received their last vaccine in the extended treatment phase within 12 months of enrolling in J09100.
- **Cohort 5**: Patients who have previously participated in the study titled "A Pilot Study of a GVAX Pancreas Vaccine (with Cyclophosphamide) in Combination with a PD-1 Blockade Antibody (Pembrolizumab) and a Macrophage Targeting Agent (CSF1R inhibitor) for the Treatment of Patients with Borderline Resectable Adenocarcinoma of the Pancreas" (IRB00130267, J1766), have no radiographic evidence of pancreatic disease recurrence, and received their last vaccine in the extended treatment phase within 12 months of enrolling in J09100.

All patients must meet the eligibility criteria including having no radiographic evidence of disease recurrence at the first vaccination and every semi-annual vaccination on this protocol.

All participants will receive 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 divided into six intradermal injections. The first vaccine boost on this study will be given no less than six months (+/- 1 month) after the last vaccination from the prior study. The vaccine boosts will then be administered every six months (+/- 1 month) until 5 years have passed and then every 12 months (+/- 1 months) until ten years have passed for Cohorts 1 and 2 or five years have passed for Cohorts 3, 4 and 5, the subject no longer meets the eligibility criteria, no longer wishes to participate in the study, or the vaccine supply is exhausted. If the last vaccination date from the prior trial has occurred more than one year ago, new semi-annual dates for vaccine boosting may be established.

In this study, research participants from Cohort 1 will remain on the same study arm as the prior study. Therefore, Arm A participants will receive the pancreatic cancer vaccine alone.

Arm B participants will be vaccinated and receive a single low-dose of cyclophosphamide (200 mg/m^2) intravenously one day prior to vaccination. Participants in Arm C will receive cyclophosphamide 50 mg once a day starting 28 days prior to day 1 of vaccination until 28 days post vaccination. Research participants from Cohorts 2-5 will be vaccinated and receive a single low-dose of cyclophosphamide (200 mg/m^2) intravenously one day prior to vaccination. The vaccine dose is the same dose found to be safe and to induce immune responses in the phase I and II studies.

If patients are also on protocol SKCCC J0248/IRB # NA_00036444 (formerly 02-10-14-03), titled "Long term follow-up of patients who received lethally irradiated pancreatic tumor cells transfected with the GM-CSF gene", the J0248 protocol will be considered inactive for them until they are off this protocol. Thus, assessments and tests included in this protocol would not be duplicated.

3.2 Study Schedule Checklist

Table 6. Prime vaccination phase for Cohort 2

	Pre-study	Each prime vaccination cycle																												
	Day 30 to Day 0	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Eligibility assessment																														
Informed consent																														
Inclusion/Exclusion[1]	X																													
Medical history	X																													
Pregnancy test	X[2]																													
Performance status	X																													
Safety assessment																														
Vital signs	X	X	X																											
Physical exam	X																													
Toxicity assessments			X																											
Laboratory test																														
Hematology[3]	X	X[5]								X[6]																				X[8]
Comprehensive[4]	X	X[5]								X[6]																				X[8]
CA 19-9	X																													
Amylase	X	X[5]								X[6]																				X[8]
Leukapheresis or blood draw (200 cc) for immune monitoring	X	X[5]																												X[8]
Blood draw for serum banking (30 cc)	X	X[5]																												X[8]
Efficacy Assessments																														
CT chest/abd/pelvis[7]	X																													
Treatment																														
IV cyclophosphamide		X																												
Vaccine[9]			X																											

In order to minimize the need for research-only in-person visits, telemedicine visits may be substituted for in-person clinical trial visits or portions of clinical trial visits where determined to be appropriate and where determined by the investigator not to increase the participants risks. Prior to initiating telemedicine for study visits the study team will explain to the participant, what a telemedicine visit entails and confirm that the study participant is in agreement and able to proceed with this method. Telemedicine acknowledgement will be obtained in accordance with the Guidance for Use of Telemedicine in Research. In the event telemedicine is not deemed feasible, the study visit will proceed as an in-person visit. Telemedicine visits will be conducted using HIPAA compliant method approved by the Health System and within licensing restrictions.

[1] Review eligible criteria for continuation.
[2] For women of childbearing potential
[3] Heme-8 with differential including absolute eosinophil count, absolute neutrophils, absolute lymphocytes
[4] Comprehensive chemistry panel including electrolytes, BUN, creatinine, SGOT, SGPT, total bilirubin, alkaline phosphatase.
[5] For prime vaccinations #2-3 only and if not done within 7 days.
[6] Can be done on day 8+/-1.
[7] Can be done within 30 days
[8] Done after prime vaccine 3 only, within +/- 7days.
[9] Prime vaccine can be administered within 28 +/- 4 days.

Table 7. Boost vaccination phase for Cohorts 1-5

	Pre-study[1]	Pre-Cycle[2]	Arm C Only	Each boost vaccination cycle																																Off Study[15]
			D-27 to D-1	0d	1d	2d	3d	4d	5d	6d	7d	8d	9d	10d	11d	12d	13d	14d	15d	16d	17d	18d	19d	20d	21d	22d	23d	24d	25d	26d	27d	28d	29d	
Eligibility assessment																																				
Informed consent	X																																			
Inclusion/ Exclusion	X																																			X
Medical history	X																																			X
Pregnancy test[3]	X	X																																		
Performance status	X	X																																		X
Safety assessment																																				
Vital signs	X			X[8]	X																															X
Physical exam	X	X																																		X
Review interval oncology notes[4]	X	X																																		
Toxicity assessments	X	X[5]		X[8]	X[9]																															X
Laboratory test																																				
Hematology[6]	X	X																																X[13]		X
Comprehensive[7]	X	X																																X[13]		X
CA 19-9	X	X																																		X

	Pre-study[1]	Pre-Cycle[2]	Arm C Only	Each boost vaccination cycle																																Off Study[15]
			D-27 to D-1	0d	1d	2d	3d	4d	5d	6d	7d	8d	9d	10d	11d	12d	13d	14d	15d	16d	17d	18d	19d	20d	21d	22d	23d	24d	25d	26d	27d	28d	29d	
Amylase	X	X																															X[13]	X
Efficacy Assessments																																				
CT chest/ abd/pelvis[14]	X	X																																		X
Treatment																																				
IV CY Cohort 1 **(Arm B) and Cohorts 2-5**[11]				X																																
Daily metronomic CY for **Arm C of Cohort** 1[12]			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Vaccine					X[10]																															
Tetanus toxoid boost[16]					X																															
Archival Tumor Tissue[17]	X																																			

In order to minimize the need for research-only in-person visits, telemedicine visits may be substituted for in-person clinical trial visits or portions of clinical trial visits where determined to be appropriate and where determined by the investigator not to increase the participants risks. Prior to initiating telemedicine for study visits the study team will explain to the participant, what a telemedicine visit entails and confirm that the study participant is in agreement and able to proceed with this method. Telemedicine acknowledgement will be obtained in accordance with the Guidance for Use of Telemedicine in Research. In the event telemedicine is not deemed feasible, the study visit will proceed as an in-person visit. Telemedicine visits will be conducted using HIPAA compliant method approved by the Health System and within licensing restrictions.

[1]Pre-Study visit to be done at least one month after candidate's last cancer treatment and up to thirty days before participants receive their first boost treatment (cytoxan or vaccine, whichever is administered first). The first boost vaccination must be no earlier than 6 months and no later than 18 months after the last prime vaccine.
[2]For Cycle 2 and thereafter, to be done within 30 days before participants receive the next boost treatment.
[3]For women of childbearing potential
[4]Reviewing pertinent interval local oncology notes including laboratory test and CT scan reports.
[5]Clinic visits not required. Research nurse calls patients to assess the toxicity on the phone. It should be done no less frequently than the following occasions: 2 months +/-15 days, 4 months +/- 15 days after the last boost treatment and within 30 days before the next boost treatment. Based on toxicity assessment, clinic visits and laboratory/imaging tests may be recommended as per standard of care and as clinically indicated.
[6]Heme-8 with differential including absolute eosinophil count, absolute neutrophils, absolute lymphocytes.
[7]Comprehensive chemistry panel including electrolytes, BUN, creatinine, AST, ALT, total bilirubin, and alkaline phosphatase.
[8]For Cohort 1 (Arm B) and Cohorts 2-5. Vital signs will be taken before IV cyclophosphamide administration. For Cycle 2 and thereafter, toxicity assessment will be assessed prior to IV cyclophosphamide administration.
[9]For all Cohorts and all cycles, toxicity will be assessed after the vaccination. For Cycle 2 and thereafter and for Cohort 1 (Arm A and C), toxicity will also be assessed prior to the vaccination.
[10]In general, 6 +/-1 months should be between two boost vaccinations. However, if participants do not meet eligibility criteria due to a reversible cause, vaccination can be postponed until they become eligible. Vaccination may also be postponed for other reasons at the discretion of the study team. The interval between two boost vaccinations is not recommended to be shorter than 5 months or more than one year. In the event of persistent vaccine-related responses, the semi-annual boost vaccination may be delayed up to one year after the last vaccine-related response.
[11]For Cohort 1 (Arm B) and Cohorts 2-5, if a patient has received IV cytoxan, but not received the vaccine on the following day for some reasons, IV cytoxan may be repeated again one day prior to the rescheduled vaccination only after the event has been discussed between PI and the study team, given that the patient remains eligible. The repeated dose of IV cytoxan must await at least 7 days after the original dose.
[12]For Cohort 1 (Arm C), vaccination should be scheduled on the day when the participant has taken 28 +/- 4 days of oral cytoxan. If the oral cytoxan is stopped with intention, it may be resumed only after the event has been discussed between PI and the study team, given that this participant remains eligible. The protocol recommends that the numbers of days of oral cytoxan prior to and post each vaccination are added together to be 56. However, if a participant misses a dose of cytoxan accidentally and without an intention, the missed dose will not be re-administrated, and a deviation should be recorded. The day of vaccination is always set as Day 1.
[13]To be completed on day 29 (+7/- days) after each boost vaccination for Cohort 1 (Arms A and B) and Cohorts 2-5, and within 7 days after completing the last dose of cytoxan for Cohort 1 (Arm C).
[14]Can be done within one month before participants receive study treatment (cytoxan or vaccine, whichever first). If allergic to the CT scan contrast, a non-contrast CT of the chest and a MRI of the abdomen and pelvis will be obtained. **Note:** Participants who have been for 5 years and more since the pancreatic surgery can have either CT scan of chest, abdomen and pelvis with IV contrast or MRI of abdomen and pelvis done at the discretion of the PI of the study following the same protocol. If MRI of abdomen and pelvis is chosen, a non-contrast CAT scan of the chest should be done.

[15]Can be done 28 days +/- 3 days of the twentieth boost vaccination cycle or within four weeks of the date when the patient is considered to be off study. Patients are considered off study beginning on 28 days after the last study treatment of the 20th boost vaccination cycle or on the day when the patient is considered to be off study prior to the twentieth vaccination.
[16]Tetanus toxoid boost if last tetanus vaccination > 10 years. Tetanus/diphtheria (Td) may be substituted if tetanus alone is not available. If both are not available, no tetanus vaccine will be administered.
[17]See Section 3.6.3. Archival tissue may be collected at any point after consent.

3.3 Study Population

One cohort of eligible patients are recruited from those who had a surgically resected and pathologically proved AJCC stage I or stage II adenocarcinoma of the head, neck, or uncinate of the pancreas (see **Section 3.2.1.1** for staging criteria) and who participated in the JHMI J0810 three-arm neoadjuvant and adjuvant pancreatic cancer vaccine trial and who have no radiographic evidence of pancreatic cancer disease recurrence (Cohort 1).

We will recruit 52 eligible patients to a second cohort from vaccine-naïve patients who had a surgically resected and pathologically proved AJCC stage I or stage II adenocarcinoma of the head, neck, or uncinate of the pancreas (see **Section 3.2.1.1** for staging criteria), and who completed adjuvant chemotherapy and/or radiation, and who have no radiographic evidence of pancreatic cancer disease recurrence (Cohort 2).

We are also recruiting patients from prior Johns Hopkins SKCCC pancreatic cancer vaccine protocols J1568, J15237 and J1766 (Cohorts 3, 4 and 5). Patients will be eligible for this arm of the study if they had a surgically resected adenocarcinoma of the pancreas (see **Section 3.3.1.1** for staging criteria), have completed the primary vaccines on the initial protocol, and have no radiographic evidence of pancreatic cancer disease recurrence.

Participants will need to be off all anti-cancer therapy for at least 28 days. In the event that a research participant has evidence of persistent vaccine-related responses (including, but not limited to: vaccine site flares such as recurrent erythema, induration, and pruritus at previous vaccine administration sites; urticaria) occurring at the frequency of more than once in the previous three months, the research participant may choose to delay the semi-annual boost vaccination for up to one year after the last vaccine-related response. The research participant may also choose to continue to receive the semi-annual boost vaccinations per protocol with evidence of persistent vaccine-related responses.

3.3.1 Eligibility Criteria

Eligibility to receive a vaccination must be determined with the first vaccination (the first prime for the Cohort 2 or the first boost vaccine for Cohorts 1, 3, 4 and 5) and then again with each semi-annual vaccination by the Principal Investigators or their designee prior to the administration of the research product. No repeated evaluation of eligibility will be done with the second and third prime vaccination for Cohort 2. However, the second and third prime vaccinations may be held at the discretion of the investigator teams or if the patient meets the off study criteria. If the eligibility criteria for vaccination are not met, the research participant may be re-evaluated if the Principal Investigators anticipates that the research participant may later meet the eligibility criteria. There is no time limit.

3.3.1.1 Inclusion Criteria for Vaccinations

Research participants must meet the following criteria:

1. Have a history of surgically resected and pathologically proven adenocarcinoma of the pancreas. Please refer to individual protocols for specific resection criteria.

2. Cohorts 1, 3, 4 and 5: Have been a participant in Hopkins IRB protocol J0810, J1568, J15237 or J1766.

3. Cohort 2: Have never received any type of pancreatic cancer vaccine/immunotherapy, had the Whipple surgery within 18 months and completed the planned adjuvant chemotherapy and/or chemoradiation.

4. Cohorts 1, 3, 4 and 5: Received the last irradiated GM-CSF transfected allogeneic pancreatic cell lines Panc 10.05 and Panc 6.03 at least 6-12 months prior.

5. Received the last anti-cancer therapy at least 28 days ago.

6. Have an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1.

7. Have provided informed consent.

8. Have adequate hematologic function (Hemoglobin ≥ 9 gm/dl, ANC ≥ 1500/cu mm, platelets ≥ 100,000/cu mm, Absolute lymphocyte count ≥ 500/ cu mm)

9. Have adequate renal function (Serum creatinine ≤ 2 mg/dl)

10. Have adequate hepatic function (Bilirubin ≤ 2.0 mg/dl, unless known Gilbert's Syndrome; AST, ALT and amylase ≤ 2x upper limit of normal: Alk Phos ≤ 5x upper limit of normal.)

11. Agree to use adequate birth control, if of childbearing potential.

3.3.1.2 Exclusion Criteria for Vaccinations

Research participants with any of the following will be excluded from study entry:

1. Radiographic evidence of pancreatic cancer recurrence.

2. Documented history of certain autoimmune diseases such as systemic lupus erythematosus, sarcoidosis, rheumatoid arthritis, glomerulonephritis, or vasculitis.

3. Uncontrolled medical problems.

4. Systemic steroid therapy within 28 days before vaccine administration with the exception of steroids utilized for the purpose of premedication for contrast CT.

5. Anticipated need for systemic steroid therapy within 28 days after vaccine administration.

6. Evidence of active infections.

7. Pregnant.

8. Have been diagnosed with another cancer or myeloproliferative disorder in the past 5 years whose natural history or treatment has the potential to interfere with safety or efficacy assessment of this study's investigational drugs.

9. History of noncompliance during previous vaccination cycles with study treatment and/or monitoring which is concerning for continued noncompliance.

3.3.1.3 Staging Information

Staging criteria are from the "American Joint Committee on Cancer (AJCC) Staging Criteria for Pancreatic Cancer Version 7." Patients with stage ≤ IIb at time of surgery are eligible for this study.

Stage Grouping

Stage Ia	T1 N0 M0
Ib	T2 N0 M0
Stage IIa	T3 N0 M0
Stage IIb	T1-3 N1 M0
Stage III	T4 Any N M0
Stage IV	Any T Any N M1

Primary Tumor (T)
TX Primary tumor cannot be assessed
T0 No evidence of primary tumor
T1 Tumor limited to the pancreas 2 cm or less in greatest dimension
T2 Tumor limited to the pancreas more than 2 cm in greatest dimension
T3 Tumor extends beyond the pancreas but without involvement of the celiac axis or the superior mesenteric artery.
T4 Tumor involves the celiac axis or the superior mesenteric artery (unresectable primary tumor).

Regional Lymph Nodes (N)
NX Regional lymph nodes cannot be assessed
N0 No regional lymph node metastasis
N1 Regional lymph node metastasis

Distant Metastasis (M)
MX Presence of distant metastasis cannot be assessed
M0 No distant metastasis

M1 Distant metastasis

3.4 Vaccine Production and Administration

[redacted]

3.5 Study Plan Schedule

Patients in the Cohort 2 (vaccine-naïve cohort) will first enter the **Prime Vaccination Phase**. During this phase, they will receive 3 vaccinations each one month apart and each in combination with a single low-dose cyclophosphamide intravenously before they proceed to the **Boost Vaccination Phase.** During the Prime Vaccination Phase, wi**th Day 1 considered as the day of vaccination and Day 0 as the day of cyclophosphamide administration, each vaccination cycle will span from Day 0 to Day 28.**

Patients in Cohort 1 (from J0810) will directly enter the Boost Vaccination Phase. During this phase, with Day 1 considered as the day of vaccination, each vaccination cycle will span from the first day when the patient receives either oral cyclophosphamide or intravenous cyclophosphamide prior to each vaccination to Day 28 or their last day of taking oral cyclophosphamide, whichever happens later.

Patients in Cohort 3, 4 and 5 (from J1568, J15237 and J1766) will directly enter the Boost vaccine phase. During this phase, with Day 1 considered as the day of vaccination, each vaccination cycle will span from the first day (Day 0) when the patient receives intravenous cyclophosphamide prior to each vaccination.

At any time during the study, additional leukapheresis or approximately 200 cc of blood may be obtained, and skin biopsies and photos may be taken of the vaccine sites and rashes, as clinical indicated, as long as the research participant is in agreement.

All research participants will be seen in the oncology outpatient center for vaccine administration and monitoring. Research participants will be monitored for at least 30 minutes following vaccination for evidence of acute reaction to the injected vaccine cells.

If not previously enrolled on SKCCC J0248/IRB # NA_00036444 (formerly 02-10-14-03) titled, “Long term follow-up of patients who received lethally irradiated allogeneic pancreatic tumor cells transfected with the GM-CSF gene,” the research participant will be encouraged to join this long-term follow-up study.

3.5.1 Pre-Study Screen

All patients on participating in Hopkins clinical protocols J0810, J1568, and J15237 (Cohorts 1, 3, and 4) who are potentially eligible will be informed of this study. Participants

in the vaccine naïve cohort (Cohort 2) will be primarily recruited based on the physician referrals and patient self-referrals. The following will occur prior to study entry for all patients:

1. Review eligibility criteria
2. An initial follow-up appointment will be made to serve as a pre-study visit.

3.5.2 Pre-Study Visit

The Pre-Study visit is to be done at least one month after candidate's last cancer treatment and up to thirty days before participants receive the first boost treatment (cyclophosphamide or vaccine, whichever is administered first) for the participants in the Cohorts 1, 3, 4 and 5. The first boost vaccination must be no earlier than 6 months (+/-30 days) after the last vaccine and within 12 months from the last vaccine for Cohorts 3, 4 and 5. For the participants in Cohort 2 (vaccine naïve cohort), the Pre-Study visit is to be done at least one month after candidate's last cancer treatment and up to thirty days before participants receive the cyclophosphamide of the first prime vaccination treatment cycle.

For all participants:

1. Obtain informed consent (if not already obtained)
2. Confirmation of eligibility (see **Section 3.3.1**)
3. A clinical evaluation to include history, vital signs, ECOG status, weight, review of systems, and physical examination.
4. CT scans of the abdomen, pelvis, and chest. If the CT scan is not done at Hopkins, the research participant must provide the report and a copy of the scans. If the research participant is allergic to the CT scan contrast, a non-contrast CT of the chest and a MRI of the abdomen and pelvis will be obtained.
5. A review of pertinent interval local oncology notes.
6. Blood testing to include CBC/diff, absolute eosinophils, absolute neutrophils, absolute lymphocytes, comprehensive chemistry panel including electrolytes, BUN, creatinine, AST, ALT, total bilirubin, alkaline phosphatase, amylase, and CA 19-9.
7. Pregnancy test for women of childbearing potential. Urine pregnancy test preferred.
8. Research Blood (Cohort 2 only): A leukapheresis for in vitro studies will be encouraged. If the research participant refuses a leukapheresis, approximately 200 cc of blood will be attempted to be drawn but may not be exact due to unforeseen technical reasons. An additional of approximately 30 cc will be drawn for serum banking. These research bloods may be drawn within 7 days of vaccination.

3.5.3 Study Procedures during the Prime Vaccination Phase (3 monthly vaccination cycles)

This is for the Cohort 2 (vaccine naïve cohort) only:

Day 0 (one day prior to vaccination)
1. Administration of cyclophosphamide 200 mg/m^2 intravenously

2. Vital signs before cyclophosphamide administration
3. For Prime Vaccination Cycle #2-3, a toxicity assessment will be done prior to IV cyclophosphamide administration.
4. Research Blood: A leukapheresis for in vitro studies will be encouraged. If the research participant refuses a leukapheresis, approximately 200 cc of blood will be attempted to be drawn but may not be exact due to unforeseen technical reasons. An additional of approximately 30 cc will be drawn for serum banking. These research bloods may be drawn within 7 days of vaccination (for Prime Vaccination Cycle #2-3 only).

Day 1 Vaccination Day
1. Administration of vaccine (Topical lidocaine-based anesthetic may be placed approximately 1-2 hours prior to planned vaccine administration time to the intended vaccine sites.)
2. Vital signs before and after vaccine administration
3. Assessment for toxicities prior to and after the vaccination.
4. Monitor for no less than 30 minutes after vaccine administration.
5. Tetanus toxoid boost if last tetanus vaccination > 10 years. Tetanus/diphtheria (Td) may be substituted if tetanus alone is not available. If both are not available, no tetanus vaccine will be administered.

Day 8 (+/- 1 day) for All Arms
1. Heme-8 with differential, including absolute eosinophil count, absolute neutrophils, absolute lymphocytes.
2. Comprehensive chemistry panel including electrolytes, BUN, creatinine, AST, ALT, total bilirubin, alkaline phosphatase, and amylase.

Day 28 (+/- 7 days)
1. Heme-8 with differential, including absolute eosinophil count, absolute neutrophils, absolute lymphocytes.
2. Comprehensive chemistry panel including electrolytes, BUN, creatinine, AST, ALT, total bilirubin, alkaline phosphatase, and amylase.
3. A research blood draw of approximately 200 cc for immune cell analysis will be attempted but may not be exact due to unforeseen technical reasons. An additional of approximately 30 cc will be drawn for serum banking.

3.5.4 Study Procedure during the Boost Vaccination Phase

This phase is for Cohorts 1, 2, 3, 4 and 5.

Pre-vaccination Assessment
The following will be done up to thirty days before participants receive each cycle of boost treatment (cyclophosphamide or vaccine, whichever is administered first) except for boost vaccine cycle #1 of the participants in Cohorts 1, 3, 4 and 5 who should have already had their pre-vaccination assessment:

1. Confirmation of eligibility (see **Section 3.3.1**)

2. A review of pertinent interval local oncology notes including laboratory tests and CT scan reports. CT scan of the abdomen, pelvis, and chest may be done within one month of boost treatment (cyclophosphamide or vaccine, whichever is administered first). If the research participant is allergic to the CT scan contrast, a non-contrast CT of the chest and a MRI of the abdomen and pelvis will be obtained **Note:** Participants who have been for 5 years and more since the pancreatic surgery can have either CT scan of chest, abdomen and pelvis with iv contrast or MRI of abdomen and pelvis done at the discretion of the PI of the study following the same protocol. If MRI of abdomen and pelvis is chosen, a non-contrast CAT scan of the chest should be done.
3. An assessment for toxicity. A clinic visit is not required. This may be done over the phone by the research nurse. Based on the toxicity assessment, clinic visits and laboratory/imaging tests may be recommended as per standard of care and as clinically indicated.
4. Blood testing to include CBC/diff, absolute eosinophils, absolute neutrophils, absolute lymphocytes, comprehensive chemistry panel including electrolytes, BUN, creatinine, AST, ALT, total bilirubin, alkaline phosphatase, amylase, and CA 19-9.
5. Pregnancy test for women of childbearing potential. Urine pregnancy test preferred.

In general, prime vaccines can be administered 28 days +/- 4 days between each vaccination while 6 +/-1months should be between two boost vaccinations. After 5 years have passed, boost vaccinations will occur every 12 months (+/- 1 month) up through year 10. However, if participants do not meet eligibility criteria due to a reversible cause, vaccination can be postponed until they become eligible. Vaccination may be postponed for other reasons including, but not limited to the occurrence of vaccine site flare-up, at the discretion of the study team. The interval between two boost vaccinations is not recommended to be shorter than 5 months or more than one year.

Day -27 to Day +28 for Arm C of Cohort 1 Only
Participants are taking cyclophosphamide 50 mg daily orally at home starting Day -27 until Day 28. For Cycle #1, prescriptions will be dispensed after meeting eligibility criteria. For Cycles 2-20, prescriptions will be dispensed on Vaccination Day of the previous cycle.

Day 0 (one day prior to vaccination) for Arm B of Cohort 1 and Cohorts 2-5
1. Administration of cyclophosphamide 200 mg/m^2 intravenously
2. Vital signs before cyclophosphamide administration
3. For Cycle #2-20, a toxicity assessment will be done prior to IV cyclophosphamide administration.

The following is applied to all participants unless indicated:

Day 1 Vaccination Day
1. Administration of vaccine (Topical lidocaine-based anesthetic may be placed approximately 1-2 hours prior to planned vaccine administration time to the intended vaccine sites.)
2. Vital signs before and after vaccine administration

3. Assessment for toxicities. For all arms and all cycles, assessment will be performed after the vaccination. For Cycle #2-20 for Arm A and C of Cohort 1, assessment will also be done prior to the vaccination.
4. Monitor for no less than 30 minutes after vaccine administration.
5. Tetanus toxoid boost if last tetanus vaccination > 10 years. Tetanus/diphtheria (Td) may be substituted if tetanus alone is not available. If both are not available, no tetanus vaccine will be administered.

Day 8 (+/- 1 day) for Cohort 2 only
1. Heme-8 with differential, including absolute eosinophil count, absolute neutrophils, absolute lymphocytes.
2. Comprehensive chemistry panel including electrolytes, BUN, creatinine, AST, ALT, total bilirubin, alkaline phosphatase, and amylase.

Day 29 (+/- 7 days)
1. Heme-8 with differential, including absolute eosinophil count, absolute neutrophils, absolute lymphocytes.
2. Comprehensive chemistry panel including electrolytes, BUN, creatinine, AST, ALT, total bilirubin, alkaline phosphatase, and amylase.

3.5.5 Off Study Visit
Patients are considered off study beginning 28 days after their final boost vaccination or the date of study termination for patients who go off study prior to their final boost vaccination or because the vaccine supply is exhausted.). Follow-up visits will be scheduled 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.

The following evaluations will be performed at the off study visit:

1. History and Physical exam with ECOG performance
2. Assessment of vaccine sites. This will include: number of sites that have erythema, induration, pruritis, and tenderness; and measurement of induration and erythema of largest vaccine site.
3. Heme-8 with differential, including absolute eosinophil count, absolute neutrophils, absolute lymphocytes
4. Comprehensive chemistry panel including electrolytes, BUN, creatinine, AST, ALT, total bilirubin, alkaline phosphatase, and amylase.
5. CA 19-9
6. CT scan abdomen/pelvis and chest. If done within 30 days the CT do not need to be repeated. (If allergic to CT scan contrast, obtain MRI). **Note:** Participants who have been for 5 years and more since the pancreatic surgery can have either a CT scan of the chest, abdomen and pelvis with IV contrast or MRI of abdomen and pelvis done at the discretion of the PI of the study following the same protocol. If MRI of abdomen and pelvis is chosen, a non-contrast CAT scan of the chest should be done.

7. Assessment of toxicities (Information may include evaluations made by the local health care provider).

3.6 Collection Samples for Correlative Studies

3.6.1 Leukapheresis

All research participants will be encouraged to undergo a standard leukapheresis for immune function analysis before each prime vaccination. Any research participant demonstrating an interesting immunological response may be asked to undergo additional leukapheresis for research purposes. This may include physical responses thought to be related to the vaccine (including, but not limited to vaccine site flares) or interesting laboratory responses (including, but not limited to mesothelin-specific CD8+T cell responses). There will be at least one month between additional leukapheresis procedures. Prior to the leukapheresis, subjects will be evaluated by the Hematopoietic and Therapeutic Support (HATS) Center to determine if their vascular access appears to be adequate for the leukapheresis procedure. If the research participant does not agree to the leukapheresis or if it is not feasible for leukapheresis, the standard 200 (+/- 10) cc of peripheral blood will be obtained. Peripheral blood mononuclear cells (PBMC) will be prepared by Ficoll-Hypaque density gradient centrifugation by standard protocol and stored at –80°C or below until further analysis. The quantity of these samples is necessary for monitoring the quantitative change of peripheral lymphocytes including Tregs and functional analysis of T cell immune response following each vaccination.

3.6.2 Serum Banking

At the time intervals indicated above, a maximum of 30 cc (and a minimum of approximately 20 cc) of peripheral blood will be drawn to collect serum from research participants for serology studies described below. Any research participant demonstrating an interesting immunological response may be asked to undergo additional serum banking for research purposes. This may include physical responses thought to be related to the vaccine (including, but not limited to vaccine site flares) or interesting laboratory responses (including, but not limited to mesothelin-specific CD8+T cell responses). The samples of serum should be allowed to set at room temperature for 20-30 minutes to allow clotting, and then centrifuged at 3,000 rpm for 10 minutes. The resultant serum will be frozen at -80°C or below.

3.6.3 Archival Tumor Tissue

Attempts will be made to obtain archived tissue samples. Archived FNA biopsy samples do not contain sufficient tissue and will not be collected. The tissue sample should have proper size to enable analysis. Formalin-fixed paraffin-embedded tissue blocks or up to 50 cut slides will be requested.

3.7 Evaluation for Safety and Anticipated Toxicities

3.7.1 Safety and Anticipated Toxicities of Vaccine

Severe toxicities are unlikely, based on information from previous GM-CSF gene-transduced whole cell vaccine studies completed here at Johns Hopkins Hospital. This includes over 200 patients treated at JHH in the Phase I and II pancreatic vaccine studies. In experiments involving over 400 mice, use of irradiated GM-CSF secreting tumor cells caused only reversible lymphadenopathy and reversible subcutaneous swelling; no ulcerations were seen. In our first phase I trial in patients with renal cell carcinoma, only local erythema and swelling were seen following intradermal injections of cell doses up to 4×10^7 GM-CSF modified vaccine cells, and up to 4×10^8 unmodified vaccine cells. At the highest dose level, we predict that initially 45 mcg of total GM-CSF will be secreted locally per 24 hours, a level that will diminish as tumor cells are killed by invading inflammatory cells. To support hematologic recovery in oncology patients after intensive chemotherapy, subcutaneous or intravenous doses of GM-CSF between 5 and 10 µg/kg/day are commonly used (350-700 mcg total for a 70 kg individual). At this dose range, the following side effects are commonly seen: local or generalized skin rashes, bone pain (attributed to stimulation of hematopoietic progenitors), fever, and malaise. Although patients in the initial Phase I study of the allogeneic tumor vaccine had normal bone marrow function, leukocytosis and toxic levels of serum GM-CSF did not occur with the 10 fold lower dose of GM-CSF. The maximum serum GM-CSF level obtained was 14.0 pg/ml with dose level four at 48 hours after the first pancreatic tumor vaccine. The plasmid used to transfect the GM-CSF gene is safe. In contrast to retroviral vectors, it lacks the coding sequences that would allow replication and the generation of helper virus. This plasmid containing the GM-CSF gene has been sequenced following vector construction to confirm its insertion, orientation, and the lack of mutations. In addition, this vector has been confirmed to produce bioactive GM-CSF.

The risk of generating autoimmune reactions is unknown but is believed to be small. The pancreas would be the most likely organ to be involved. Pancreatitis and loss of pancreatic function can be supported by the use of exogenous pancreatic enzymes and insulin injections if needed. Other organs that may share tissue specific antigens might also be involved, such as the salivary glands and other gastrointestinal organs. In the Phase I and II studies there were no evidence of autoimmune reactions. Every patient who has received the vaccine will be evaluated for toxicity. The research participant will be taken off-study if unacceptable adverse events are experienced. Possible toxicities include local swelling, induration, or ulceration at the site of the vaccine, systemic toxicities from paracrine secretion of GM-CSF, and induction of autoimmunity. The risk of generating autoimmune reactions might be increased by combination of cyclophosphamide; however, no autoimmune reactions have been reported in previously published studies of metronomic cyclophosphamide. Therefore, we anticipate that such a risk is still small even if cyclophosphamide is combined with the vaccine.

Blood tests to monitor for systemic toxicities will be obtained a week and four weeks after all vaccinations. For the first vaccination, blood tests for systemic toxicities will additionally be performed two, three, and four months after vaccination All research participants will be followed for cancer recurrence every three to six month by their local

health care providers or as determined by each provider. The research participant may be contacted by phone or e-mail or the information may be obtained from their local health care providers. The research nurse will contact patients to assess the toxicity by phone. It will be done no less frequently than the following occasions: 2 months +/-15 days, 4 months +/- 15 days after the last vaccination and within 14 days before the next study treatment. Based on toxicity assessment, clinic visits and laboratory/imaging tests may be recommended as per standard of care and as clinically indicated. The research participant will be advised to call the research nurse and/or the principal investigator if there are any new toxicities, concerns or questions.

3.7.2 Safety and anticipated toxicities of single injection of low dose cyclophosphamide or recurrent administration of metronomic cyclophosphamide

The dose of cyclophosphamide studied in this trial is below that in common use for the adjuvant therapy of e.g. breast cancer (typically 600 mg/m^2 of cyclophosphamide) (Sledge, 1998). Therefore, we anticipate that the risk of toxicity related to the use of single dose of cyclophosphamide at 200 mg/m^2 or that of daily metronomic cyclophosphamide at 50 mg once daily eight weeks is quite small. Based on the toxicity studies of higher dose of cyclophosphamide in adjuvant therapy of breast cancer, fatigue, alopecia, nausea, vomiting, and mild cytopenias are likely to be the most common toxicities of cyclophosphamide. With standard dose cyclophosphamide, a rare incidence of leukemia or myelodysplastic syndrome as a late toxicity was reported.

Long-term daily use of cyclophosphamide may have different toxicity profiles from higher dose of cyclophosphamide given intravenous every 2-3 weeks. Nonetheless, animal studies still suggest that continuous administration of metronomic dose of cyclophosphamide is significantly less toxic than the maximum tolerated dose of cyclophosphamide given intermittently for 3 doses (Emmenegger et al., 2004). The toxicities of metronomic cyclophosphamide in humans can be anticipated based on the previously published studies. Orlando et al. (2006a) reported in patients with metastatic breast cancer that prolonged treatment of metronomic cyclophosphamide at 50 mg/day for a median duration of treatment of 20.4 months was well tolerated and side-effects were mild. The most frequently encountered toxicity was grade 1-2 leukopenia, which was observed in 54% of the 63 cases. Increases in transaminase values were registered in 12 cases, and one patient had grade 3 toxicity. Other side effects included one patient with grade-3 thrombocytopenia, five with grade 1-2 anemia, ten with grade-1 and one with grade-2 nausea/vomiting, and small percentages of patients with grade 1-2 mucositis, gastric pain, diarrhea, fever, infection, asthenia, etc. It should be noted that patients in this study were also given metronomic dose of methotrexate which likely resulted in some of the side effect profiles Other studies reported similar toxicity profiles with prolonged use of metronomic cyclophosphamide (Orlando et al., 2006b; Glode et al., 2003; Bottini et al., 2006; Suvannasankha et al., 2007). The dose and schedule of metronomic cyclophosphamide in this study is adopted from Ghiringhelli et al. (2007), where the short-term and long-term safety was shown.

Long-term use of metronomic cyclophosphamide has the potential to be associated with leucopenia, thrombocytopenia, infection, transaminitis, and hemorrhagic cystitis. The most commonly anticipated toxicity due to cyclophosphamide is leukopenia. However, most cases of myelosuppression induced by metronomic cyclophosphamide are reversible with dose reduction or temporary discontinuation (Austen et al., 2001, O. de Weerdt et al., 2001, Lord et al., 2007). For intravenous administration, the nadir in the white blood cell count occurs between 8-14 days after starting treatment with a full recovery 21 days after administration (Austen et al., 2001). The degree of lymphopenia and the length of time to white blood cell count nadir are dose-dependent. The time course of hematologic effect in daily oral cyclophosphamide is less predictable but based on other metronomic cyclophosphamide studies, anemia, neutropenia and thrombocytopenia occur less commonly and to a lesser degree than leukopenia (Lord et al., 2007). The development of opportunistic infections is associated with the degree and duration of leucopenia. The risk of infection increases when the white blood cell nadir decreases to below 3000 cells/muL or the absolute neutrophil count falls below 1000 cells/muL (Fox and Pandya, 2000). The most common opportunistic infection is Pneumocystis carinni pneumonia, which can be prevented with low-dose trimethoprim-sulfamethoxazole (Beimler et al., 2004).

Hepatotoxicity is known to occur with high dose cyclophosphamide, but is rare at low doses. In the cases that hepatotoxicity did occur, liver injury occurs within 2-8 weeks of treatment and liver function normalized after discontinuation of cyclophosphamide (Mok et al., 2000, Akay et al., 2006). The risk of hemorrhagic cystitis is dose dependent and due to accumulation acrolein, a cyclophosphamide metabolite. It may occur during treatment or months after treatment. The risk of bladder toxicity is higher with oral metronomic doses than intermittent intravenous doses based on studies with lupus nephritis, vasculitis, and Wegener's granulomatosis patients. However, we expect rare, if any, cases of hemorrhagic cystitis since it is associated with higher cumulative doses of 57 to 100 g (Fox and Pandya, 2000). As reported, essentially all the cases of hemorrhagic cystitis induced by long-term low dose cyclophosphamide treatment were reversible with appropriate treatments.

3.8 Interval Health Care

The research participant disease status may be evaluated more frequently than twice a year. Patients may undergo additional abdomen, chest and pelvis CT scans and blood tests, including CA 19-9 levels, as recommended by their local oncologist, or by the study team as standard of care for possible disease recurrence. The results of the CT scans, blood tests, and progress notes will be requested from the research participant's local oncologist. Medical records obtained will be added to the oncology outpatient medical records, including electronic forms, when possible. Data related to the toxicity and/or disease status assessments will be recorded in the case report form binders and electronic database. If the research participant is followed at Johns Hopkins, the medical records will be accessed at least quarterly, and more frequently, if needed for disease recurrence.

If patient is taken off study, the patient's medical records will be requested from the health care provider providing further care for 28 days after the last research product was administered, or longer for resolution of any adverse events related to the research product.

After off-study, the research participant will be followed annually via the protocol SKCCC J0248/IRB # NA_00036444 (formerly 02-10-14-03) titled, "Long-term follow-up of patients who receive lethally irradiated allogeneic pancreatic tumor cells transfected with the GM-CSF gene", if consent is granted.

3.9 Termination of Study Treatment (Off Study)

Patients in Cohort 1 and 2 will be considered to have completed the study when they have received 10 years of treatment. Patients in Cohort 3, 4 and 5 will be considered to have completed the study if they have received all ten boost vaccinations. Every attempt will be made to schedule an off-study visit within 4 weeks of the date of study termination for patients who were unable to complete the 10 years of treatment for Cohort 1 and 2 or ten vaccinations for Cohort 3, 4 and 5. If determined to be off-study, the research participant will be followed for 28 days after the administration of the last vaccine for toxicities related to the vaccine, or longer, if vaccine-related toxicities occur.

The patient may be discontinued from treatment or participation in the study in the following instances:
1. Patient withdraws consent and refuses future vaccinations
2. Patient is repeatedly noncompliant with study treatment
3. Concurrent illness develops that would preclude objective clinical assessments
4. The incidence or severity of adverse events in this study denotes potential untoward health risk to the patient.
5. If the patient is diagnosed with autoimmune diseases such as systemic lupus erythematosus, sarcoidosis, rheumatoid arthritis, glomerulonephritis, or vasculitis.
6. Patient receives non-study immunotherapy, chemotherapy, radiotherapy, gene therapy, biologic therapy, or other investigational therapy for the treatment of pancreatic cancer.
7. Patient experiences a dose-limiting toxicity attributed to study vaccine. Dose-limiting toxicity (DLT) is defined as any grade 3 or 4 non-hematological toxicity excluding alopecia, grade 3 hematologic toxicity, and grade 4 hematological toxicity that does not resolve in less than 5 days. If a DLT (except alopecia) occurs, treatment will be stopped. Treatment may be restarted if the DLT resolves to < grade 2 or to baseline levels. If the toxicity continues at > grade 2 for four weeks then the patient will be removed from further treatment in the study.

Per the FDA guidance for patients treated with genetically modified products, all research participants will be encouraged to enroll in a long-term follow-up protocol, following their completion of all interventional studies. These patients will be followed for disease progression, survival and potential long term toxicity of gene therapy in an existing protocol titled "Long term follow-up of patients who received lethally irradiated allogeneic pancreatic tumor cells transfected with the GM-CSF gene (SKCCC J0248/IRB # NA_00036444 [formerly 02-10-14-03])".

3.10 Management of Toxicities

Local vaccine site reaction may be treated with topical applications of aloe vera or vitamin E gel or lotion. Significant local inflammation that is causing the research participant severe pain or is interfering with the activities of daily living may be treated with cold packs and oral analgesics. Local toxicities of pruritus at the vaccine sites and systemic pruritus may be treated with topical or oral diphenhydramine hydrochloride (Benadryl) or topical aloe vera. If oral diphenhydramine hydrochloride is used the recommended dose shall be 25-50 mg every four to six hours as needed for pruritus, not to exceed 300 mg/day. Cases of local ulceration should be manageable with local wound care, with or without antibiotics. Severe local inflammation or significant clinical autoimmunity will be managed on a case-by-case basis.

For patients with grades 3 and 4 leukopenia, neutropenia or thrombocytopenia who are due to receive IV cyclophosphamide , both the IV cyclophosphamide and vaccine will be held until the patient's counts return to grade 2 or above. The patients will be taken off the study if their counts do not return to grade 2 or above within 6 months during the prime vaccination phase or within 6 months during the first five years or within 12 months in after five years during the boost vaccination phase. For patients with Grade 3 or 4 leukopenia, neutropenia or thrombocytopenia who are still receiving oral cyclophosphamide treatment, treatment can be restarted once the myelosuppression has returned to grade 2 or above. If the myelosuppression has not recovered to grade 2 by the time of the next scheduled vaccination, both the oral cyclophosphamide and vaccine will be held until the patient’s count return to grade 2 or above. The patients will be taken off the study if their counts do not return to grade 2 or above within 6 months during the prime vaccination phase or within 6 months during the first five years or within 12 months in after five years during the boost vaccination phase. Prophylactic doses of trimethoprim-sulfamethoxazole will be recommended to all patients with Grade 3 or 4 lymphopenia or neutropenia lasting more than a month at the discretion of the treating physician. If the patient is allergic to trimethoprim-sulfamethoxazole, dapsone will be recommended as an alternative.

Patients receiving cyclophosphamide who develop hematuria or polyuria will undergo urinalysis and cytology. If hemorrhagic cystitis is identified, oral or IV cyclophosphamide will be discontinued and appropriate treatments will be initiated as per standard of care. Such patients may receive their scheduled vaccination at the discretion of the study team and appropriate surveillance will be performed for transitional cell carcinoma of the bladder.

3.11 Concurrent Medication

Concurrent medications will be collected as a part of “Medical History” when it is taken as indicated in **Section 3.2** - Checklists and **Section 3.5** - Study Visit Procedure. In addition, concomitant medications will be recorded during each vaccination cycle from Day 0 to Day 28 for Cohort 1 (Arm A and Arm B) and Cohorts 2-5 and from Day -28 to Day 28 for Cohort 1 (Arm C). No other chemotherapy, immunotherapy, radiotherapy, gene therapy, biologic therapy, or other investigational therapy for the treatment of pancreatic cancer is allowed during the course of this study. Systemic corticosteroids or other

immunosuppressive drugs should be avoided for 28 days before and after each vaccination. Local steroid treatments are allowed.

4.0 Statistical Considerations

4.1 Sample Size

The primary statistical endpoint of this study is to determine the safety and feasibility of vaccine boosting with lethally irradiated, allogeneic pancreatic tumor cells transfected with the GM-CSF gene given alone or in combination with either a single intravenous dose or long term daily metronomic oral doses of cyclophosphamide. The sample size of Cohorts 1, 3, 4 and 5 is predetermined by the prior parent studies J0810, J1568, J15237 and J1766. The J0810 study will have enrolled approximately 60 participants. Among them, 39 are evaluable subjects who have received at least 3 vaccines. For patients who receive all six vaccinations under the J0810 protocol, the first boost vaccination will be scheduled around 20 months following surgery. Based on analysis from the phase II adjuvant study (Laheru et al., 2007), we anticipate the disease free survival rate at 20 months to be approximately 40%-50% respectively. Therefore, we estimate that up to 50% of these 39 evaluable patients, equal to approximately 20 patients, will be candidates for this boost vaccination study. It should be noted that some participants from the J0810 may have completed less than three vaccination cycles and then been taken off the study due to reversible conditions, and may again become eligible for this boost vaccination study. Nonetheless, we estimate that such subjects only account for less than 5% of the total participants in the J0810 study.

We have recognized the power of this study would be limited by the sample size that is predetermined by the original J0810 study size. To adequately power this study to evaluate the immunologic endpoint, we add a vaccine-naïve cohort. Based upon the previous J0619 boost vaccine trial, 70% of the patients remained disease-free and completed their first follow-up boost. In addition, in the previous J9988 study, 50% of patients who were tested showed a positive response (defined as $CD8^+$ T cells of two high-avidity mesothelin epitopes with enhanced functional activity) following their first 6-month boost vaccine. A sample size of 26 would provide 90% power to detect an increase from the null rate of 50% to 80% with a two-sided type I error rate of 5%. Based upon the previous J0619 boost vaccine trial, 70% of the patients remained disease-free and completed their first follow-up boost. Therefore, we initially estimated that we needed to enroll a total of 38 individuals to allow for the 30% loss to follow-up. In addition to the 20 patients that were estimated for the above J0810 cohort, we originally estimated to enroll a total of 58 patients to this study. However, based on the preliminary enrollment data of this current study, approximately 50% patients went off the study prior to receiving the first boost vaccine. More patients went off the study due to disease recurrence was because of more frequent use of CT scan as a surveillance in today's practice than when the previous J0619 study was conducted. Another reason is that all the patients enrolled in this study were within 18 months out of the surgery and thus had a high risk of recurrence than those enrolled in the J0619 study. After considering the preliminary enrollment data from this current study. We re-estimated the sample size of the vaccine-naïve cohort to be 52. Therefore, we

estimate the total sample size of this study including both the J0810 cohort and the vaccine-naïve cohort to be 72.

We still lack data to distinguish the immune modulatory effects of these two different routes of cyclophosphamide administration. Since the single IV dose of cyclophosphamide has been used more commonly in the past, we have chosen to add a new cohorts with the single IV dose of cyclophosphamide (Cohorts 3-5), which will allow for a better comparison to historical cohorts. It should be noted that the sample size of the new cohorts is powered separately, but this new cohorts will not be analyzed separately. Therefore, the data analysis part of the protocol remains unchanged. All cohorts will receive the boost vaccinations in the same way, which is the main study procedure of the protocol. The main objective is also the same, to study the effect of the vaccine boosting. Nonetheless, we have to calculate the sample size of the vaccine cohort separately because the sample number in Cohorts 1, 3, 4 and 5 cannot be estimated precisely. The sample number in Cohorts 1, 3, 4 and 5 depends on how many patients remain disease-free after they complete the primary studies and how many are interested in the boost vaccination. When we calculate the sample size of the new cohort separately, we can ensure that the sample size of this study has the sufficient power to compare with the historical control patients, those treated in the J0619 study – the previous boost vaccine study. In addition, there is a small difference between how each cohort received the prime vaccinations. Cohort 2 will receive the prime vaccination in a similar way to the patients in the J0619 study (historical comparison cohort). By calculating the sample size of the new cohort separately, we can perform a more formal comparison between patients in this study and those in the J0619 study. Certainly, we will also combine the patients in all cohorts for comparison and combine Arm B of the J0810 cohort (Cohort 1), and vaccine-naïve cohort (Cohort 2) for comparison. We estimate the sample size of Cohort 3, 4 and 5 to be 68.

4.2 Analysis of Primary Endpoints

4.2.1 Safety and Toxicity Measurements

The primary endpoint of this study is safety as measured by local and systemic toxicity. For this endpoint, all the patients who are enrolled in the study will be evaluated. These toxicities will be characterized according to the National Cancer Institute Common Terminology Criteria for Adverse Events, Version 3.0, and can be accessed and downloaded via the website: http://ctep.cancer.gov/reporting. Local dermatologic reactions anticipated according to the previous studies of the same vaccine are known to be appropriate vaccine-induced immune responses (Jaffee et al. 2001), therefore, are not considered toxicities, adverse events or problems, and will no longer be reported. Plenty of scientific information has been collected by the previous studies; thus, measurements of the vaccine sites and routine skin biopsy will not be performed. Nonetheless, unusual types of vaccine site reaction including but not limit to ulceration will be recorded. An optional skin biopsy and/or photographs may be obtained if the subject has a systemic rash, or an unusual vaccine reaction. In addition to sending this to pathology for diagnosis we will use additional material from the biopsy for research purposes to understand the effects of the vaccine. For each participant arm, we will tabulate the number, type and degree of toxicities

for each round of vaccination. In addition, we will estimate the proportion of individuals who have a DLT within the first 28 days of vaccination for each cycle with an exact 95% confidence interval. We will estimate the frequency of each toxicity per total number of patients treated (total number of treated patients will be included in denominator), as well as per total number of vaccines that are administered (total number of vaccines will be included in denominator).

We expect to be able to estimate the frequency of the toxicities that were initially identified in the phase I and II studies that are vaccine related. Based on the phase I data, we do not expect to detect significant local or systemic toxicities. The toxicities of low-dose cyclophosphamide have also been well characterized. The toxicity profile of the combination of low-dose cyclophosphamide and vaccination can be estimated from the above-described phase II study in patients with metastatic pancreatic cancers. Because pancreatic adenocarcinoma 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).

In addition, although it is unlikely that life-threatening toxicities will be uncovered in this study, a single life-threatening adverse event would suspend accrual. Then, a complete evaluation of all other observed adverse events to define their severity and to determine the degree to which each event can be definitively attributed to the vaccine treatment will be warranted. Based on the results of this evaluation, modifications in the form of dose reduction, schedule change, or vaccine lot replacement would be made in order to resume clinical testing.

4.2.2 Measurements of Immune Response

We will measure the number, repertoire and avidity of peripheral mesothelin-specific $CD8^+$ T cells. When these parameters of immune response are measured, continuous variables will be summarized with means and standard deviations. Dichotomous and categorical variables will be summarized using proportions with exact 95% confidence intervals and counts, respectively. These summaries will be computed for each vaccinated patient at multiple time points before and after vaccine administration: pre-vaccination and four weeks post vaccination (also as indicated in Study Plan). Plots will be used to show the changes in immune response over time both for each individual and for each arm. For each vaccination, comparisons in the pre and post-vaccine responses will be compared using paired t-tests (or Wilxcoxon signed rank tests if appropriate) for continuous variables and McNemar's test for dichotomous or categorical variables. Associations between immune responses will be explored graphically (e.g. scatterplots, boxplots) and numerically (e.g. correlations, χ^2 tests).

Since the initial therapies differ between the four cohorts, we will consider comparing each cohort against a historical control, respectively. Therefore, while the sample size of Cohorts 1, 3, and 4 is small and predetermined, the sample size of Cohort 2 (the vaccine-naive cohort) is powered separately. We will also consider merging the following cohorts to compare against the historical control: Cohort 1 (Arm B), Cohort 2, Cohort 3 (Arm A),

Cohort 4 and Cohort 5. We will also compare between the two cohorts although it is limited by the small sample sizes.

One of the main goals of this study is to determine whether or not the boost vaccination scheme is safe and feasible. If the study treatment is not feasible, we would never be able to answer the immunologic questions. In addition, till today, none of long-term immune response parameters have been accepted as the primary endpoints. Therefore, we will still keep the safety endpoint as the primary endpoint.

4.3 Analysis of Secondary Endpoints

4.3.1 Efficacy Endpoints

The secondary efficacy endpoint of interest is the overall survival (OS) of patients treated with vaccine. The overall survival is defined as the time from initial randomization for the J0810 study until death. If a patient is lost to follow-up or the study is ended prior to death, the patient will be considered censored at their last recorded follow-up. For each arm, Kaplan Meier curves will be constructed after combining all patients from three arms and the median survival estimates will be calculated with 95% confidence intervals using Greenwood's formula. All participants will be evaluable for efficacy endpoints.

We are also interested in investigating the effect of vaccine on disease/progression free survival (PFS). Patients who are treated at Johns Hopkins will be followed every 3 months and evaluated for recurrence with the CA19-9 level and the CT scan, as recommended by the NCCN guideline. However, the standard of care follow-up, such as the interval of follow-up and the type of follow-up imaging, varies from institution to institution. PET scan is not typically done as a follow-up image for resected pancreatic cancer. However, if a PET scan has been done and its result is available, we may use the results of PET scan to help determining the recurrence by following the guidance of RECIST 1.1. In order to prevent biasing our results, it is necessary to coarsen the follow-up interval and the selection of imaging type so that every individual is evaluated in the same manner regardless of the institution. This coarsening of the data may cause an overestimation of PFS. In order to take into account both potential forms of bias, we will perform our analysis of PFS on the entire cohort as well as the subpopulation of patients from Johns Hopkins (estimated to be approximately 50% of the research participants). PFS will be defined as the time from the first vaccine until evidence of disease recurrence. If a patient withdraws from the study prior to being diagnosed with progressive disease, they will be censored at the date of their last follow-up visit. If an individual dies prior to being diagnosed with progressive disease, they will be considered to have progressed at the date of their last follow-up visit. This is a conservative estimate if we count the disease recurrence at the next planned visit then this will bias the PFS time upward. The PFS will be summarized using Kaplan-Meier curves and estimates of median PFS with 95% confidence intervals based upon Greenwood's formula. Due to potential causes of bias discussed earlier, the results will be considered extremely preliminary. If the PFS estimates for patients treated at Johns Hopkins appear to be lower than that of the population as a whole and the Johns Hopkins patients are followed at more frequent intervals than the other patients, then this

indicate that an overestimation of PFS may exist. No formal comparison is planned due to the small sample size. Such results could affect the future planning of follow-up treatment for patients in subsequent trials.

Although cyclophosphamide administrated in two different ways will be tested in different arms, the primary endpoint is to evaluate their safety, feasibility and immune responses including mesothelin-specific T cell responses and Treg quantity and function. Our second endpoint is to evaluate whether cyclophosphamide administrated as in Arm B and Arm C would have significant impacts on clinical efficacy. However, even if cyclophosphamide administration would have a significant impact on clinical efficacy, we do not anticipate that the sample sizes will be adequately powered to compare the clinical efficacy between arms. The purpose of different design is to discern the difference in anti-tumor immune responses when cyclophosphamide is administrated in a different way. The data will serve as the rationale for the choice of cyclophosphamide administration schedule/dose in the future clinical studies. For instance, if one modality of cyclophosphamide administration results in a more favorable immune response, we will postulate that such a schedule of cyclophosphamide administration would also result in a more favorable clinical response. We will also combine all three arms and compare them with data from our prior phase II adjuvant study or historical data of non-vaccinated patients.

4.3.2 Relating Immune Response to Clinical Response

The relationships between immune and clinical responses will be assessed using a variety of statistical techniques. Preliminary explorations will be graphical in nature (e.g. boxplots, scatterplots). Univariate and multivariate modeling will be used quantify the associations. In the case of a binary clinical outcome (e.g. toxicity), logistic regression will be used. In the case of a time-to-event clinical outcome (e.g. OS), the Cox proportional hazards model will be used.

4.4 Analysis of Exploratory Endpoint

Genomic sequencing library construction, whole genome/exome sequencing, whole transcriptome sequencing, microbial sequencing, neoepitope prediction, mutation burden, and bioinformatic analysis may be performed either at an on-campus laboratory or at an off-campus sequencing service. All the samples will be de-identified before sending to any laboratory for sequencing. The FASTQ files, BAM files and VCF files will be generated and analyzed. Other sequencing assays may be performed on a subset of samples according to specific requirements of collaboration projects.

Results from the sequencing studies will not be released to the patients. These studies are for research purposes only and are not using a clinically validated platform.

Genomic sequencing data will be either destroyed or stored on a JHU managed, HIPAA-compliant, password protected hard drive or Johns Hopkins University School of Medicine PMAP.

Genomic sequencing data will be either destroyed after the research or stored and computations in a HIPAA-compliance space.

5.0 Response Criteria

5.1 Evaluation of Clinical Activity

Most patients will be expected to have only minimal residual 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 this trial will be compared among different arms, to the results of our prior phase II adjuvant study, to historical controls seen at the Johns Hopkins Hospital. Patients recently seen at the Johns Hopkins Hospital who can be matched for pathologic stage, surgical intervention, and adjuvant combination chemotherapy and radiation therapy, are the most accurate group of historical controls since our institution has the largest reported experience treating patients with this disease and has recently reported the best survival statistics for current interventions. Patients may 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 local or metastatic recurrence by CT scan. The serum tumor marker CA19-9 lacks a sufficient sensitivity and specificity to serve as reliable indicators of response. 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.

5.2 Evaluation of Immune Responses

A central goal of the clinical trial is to identify immunologic changes associated with the vaccine therapy that may be markers of potential clinical responses. Studies include, but are not limited to, the analysis of mesothelin-specific antitumor immune responses. All the techniques involved are well established at Johns Hopkins, and these studies will be performed in close collaboration with appropriate CORE facilities.

The leukapheresis product or peripheral blood will be obtained from each research participant at periodic protocol-specified intervals for the in vitro assays. Serum will also be obtained at the same intervals for humoral immune response. In addition, research participants may be asked to donate additional lymphocytes by leukapheresis and additional serum. Informed consent will be obtained for this procedure using the Leukapheresis Informed Consent Form, which is a separate document than the main vaccine study informed consent form.

6.0 Adverse and Problem Event Reporting

6.1. Responsibilities

It is the responsibility of the principal investigator to notify the IND sponsor of the vaccine research product, Elizabeth Jaffee, M.D., Hopkins Medicine Institutional Review Board, and Hopkins Institutional Biosafety Committee of any serious adverse event due to any cause, which occurs during the course of this investigation, and is believed to be in any way related to study drug. The sponsor will notify the appropriate federal regulatory agencies, including but not limited to the Food and Drug Administration. The Principal Investigator or their designees must notify the IND sponsor, Elizabeth Jaffee, M.D. of any Serious Adverse Event (SAE) within 24 hours of the investigator learning that the adverse event has occurred. Events must be documented on the Johns Hopkins Medicine IRB form titled, "Problem/Event Report Form" available at **http://irb.jhmi.edu.** See section 6.3 for IRB reporting guidelines.

All adverse and problem events as well as serious adverse events occurring from day 0 to 28 of each vaccine cycle will be recorded.

A *serious adverse event (SAE)* is one that:

- Results in death
- Is life-threatening
- Requires inpatient hospitalization or prolongation of an existing hospitalization > 24 hours
- Results in persistent or significant disability/incapacity
- Is a congenital anomaly/birth defect in the offspring of an exposed patient

An important medical event that may not result in death, be life-threatening, or require hospitalization, may be considered a serious adverse drug experience when, based upon appropriate medical judgment, it jeopardizes the subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.
A *life threatening adverse event* is defined as any adverse experience that places the patient or subject, in the view of the investigator, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that, had it occurred in a more severe form, might have caused death.
An *adverse drug reaction (ADR)* is a noxious and unintended response to a medicinal product in which a causal relationship between a medicinal product and an adverse event is at least a reasonable possibility, i.e. that the relationship cannot be ruled out.
A planned hospitalization however is not considered a SAE.
An elective surgery will be considered an AE but not an SAE unless the hospitalization is prolonged due to complications.

6.2 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 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.

All adverse events will be recorded in the subject's Case Report Form and in the study database. 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 3.0 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.
- ***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.

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, except for lymphopenia, neutropenia and leukopenia should be reported as an adverse event only if it is considered clinically significant by the investigator. Regular vaccine site reactions such as erythema, swelling, indurations, pruritus and tenderness will be noted by the RN in each visit note but will not be reported as an AE. However, reactions outside the vaccination site such as hives and rashes or any significant reaction due to vaccination will be reported as an AE.

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.

6.3 Reporting 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.

7.0 Clinical Trial Monitoring

On a regular basis, the protocol will be internally monitored by the principal investigator, and the study's sponsor, Dr. Elizabeth Jaffee.

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.

8.0 Developmental Methods

The following projects have been developed with the goal of improving the specificity and efficacy of this GM-CSF transfected whole tumor pancreatic cell vaccine. These methods should be considered developmental.

8.1 General Rationale for Banking Patient Materials

Recent data from human melanoma studies have revealed that the majority of tumor-specific antigens identified so far are shared among MHC-matched melanoma tumors. In addition, the cellular proteins from which they derive are often shared among non-MHC matched tumors. Preliminary studies of human renal cancer have demonstrated similar findings (Zhou et al., 2005). We have begun to address this question in patients with pancreatic adenocarcinoma using patient materials from the phase I and phase II studies. The demonstration of shared antigens will drive an intensified effort to identify these antigens for the purpose of developing future, antigen-specific vaccines, thereby eliminating whole cells as a source of antigen. In particular, these antigens could then be employed in novel strategies that take advantage of our more recent understanding of tumor antigen processing and presentation mechanisms, hopefully leading to enhanced immune priming beyond that which can be achieved with cellular vaccines. The following materials will be collected for these developmental goals.

8.2 Serum Banking

Serological analyses (SEREX or SERPA) are commonly used to identify tumor-associated antigens or antigens targeted by T cells (Nishikawa et al., 2005; Zhou et al., 2005). To establish a both reliable and feasible way to predict anti-tumor immune response and also to identify novel pancreatic tumor antigens, Dr. Jaffee's laboratory has used the serum from patients who have shown anti-tumor immune response in the previous phase II clinical trials to develop a functional proteomic approach to screen the antigens targeted by the antibody responses induced by vaccination (unpublished data). The whole cell extract from Panc 6.03 and Panc 10.05 cells were used as the proteome and fractioned by chromatography and chromatographic fractions of proteins were further separated by 2D protein electrophoresis. They were then subjected to immunoblotting analysis by using patients' sera to identify the antigens that can be recognized by the antibodies in the sera. The immunoblots with the serum from patients prior to vaccination was compared with those with the post-vaccination serum. The proteins that were unique in the immunoblots with the serum from post-vaccination patients were sequenced by mass spectrometry and their entities were identified. These proteins, representing the antigens that are targeted by vaccine-induced humoral immune response, comprise a proteomic array that can be used to predict anti-tumor immune responses.

This trial will provide us with an opportunity to begin to validate the vaccine-induced antibody responses to these antigens and the values of the proteomic array comprised of these antigens in predicting anti-tumor immune responses. In this study, we will bank the serum from each participant prior to and following each vaccination. As noted above, approximately 20-30 ml of blood will be required at these time points as well as at other indicated time points for serum banking. They will be used in the immunoblot analyses of individual vaccine-specific antigens or in the ELISAs to assess whether antibodies to these antigens are specifically induced by vaccination. Once the analysis of clinical efficacy is completed, the induction of vaccine-specific humoral immune response, reflected by the presence of vaccine-specific antibody production in serum, will be correlated with patients' clinical response and T cell immune response.

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