Section 2: Scientific Abstract

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

BACKGROUND AND PRELIMINARY RESULTS

Cancer of the pancreas is the tenth leading cause of cancer in the United States with an estimated incidence of 37,170 new cases in 2007. It is also one of the most lethal malignancies and is currently the fifth leading cause of cancer death with an estimated number of deaths in 2007 similar to the incidence rate. The death rate in pancreatic cancer is exceeded only by lung, colorectal, breast, and prostate cancer. Despite recent advances in the overall understanding of pancreatic cancer at the molecular level, improved imaging techniques to identify disease at an earlier stage, improved surgical techniques as well, as a growing body of literature that supports the role of adjuvant therapy, the 1-year survival is still on the order of 20%, with a median survival of 15 to 19 months for resectable disease and a 5-year survival of approximately 3% for all stages combined. Only two drugs, Gemcitabin and Erlotinib, are currently approved for this disease. These drugs were approved by the FDA based on a significant improvement in quality of life and a 2-week survival benefit, respectively.

Immunotherapy is an innovative approach being developed for the treatment of pancreatic cancer, a relatively chemotherapy-resistant disease. The ideal vaccine would provide shared immunodominant tumor antigens that could subsequently recruit and activate tumor specific CD8$^+$ and CD4$^+$ T cells. We have developed an irradiated gene modified allogeneic pancreatic cancer vaccine and have presented safety data and survival data using this vaccine in the adjuvant setting integrated with chemoradiation in an exploratory vaccine dose finding phase I study (Jaffee et al., 2001) and subsequently in a follow-up phase II study (Laheru et al., 2007; Laheru & Jaffee, et al., manuscript in preparation). In addition, we have combined the vaccine with immune modulating doses of Cyclophosphamide (Cy) in the metastatic setting (Laheru et al., Clin Ca Res, 2008). In all three studies, we have shown the induction or enhancement of mesothelin-specific CD8$^+$ T cell responses which correlates with survival (Thomas et al., 2004; Laheru et al., 2008; Laheru & Jaffee, et al., manuscript in preparation). Additionally, patients with longer overall survival not only develop an increase in the number of mesothelin-specific T cell responses but an enhancement in the quality of the T cell response as determined by tetramer analysis of T cell avidity.

One of the obstacles to effective immunotherapy is the induction of immune tolerance to cancer cells. T regulatory cells (Tregs) play a pivotal role in impeding this obstacle. A single intravenous dose of Cy has been commonly proposed as an immune modulator to deplete Tregs when it is in combination with experimental immunotherapy; however, animal studies suggested it does not have a durable effect in depleting T regulatory cells (Ercolini et al., 2005). While several agents including anti-CTLA antibodies are under development to modulate immune tolerance in order to enhance the
immune response, repetitive administration of metronomic Cy has been shown to lead to durable inhibition of T regulatory cells (Ghiringhelli et al., 2007). This proposal aims to build on our experience with the GM-CSF secreting pancreatic cancer vaccine by combining the vaccines with Cy, particularly the metronomic Cy, and our experience with this combination to treat patients with resectable pancreatic adenocarcinoma in both neoadjuvant and adjuvant setting.

**OBJECTIVES**

**Primary:**
1. To evaluate the safety and feasibility of administration of a lethally irradiated, allogeneic pancreatic tumor cell vaccine transfected with the GM-CSF gene alone, or given with either a single intravenous dose or daily metronomic oral doses of cyclophosphamide for the treatment of patients with surgically resectable adenocarcinoma of the head, neck, or uncinate process of the pancreas as neoadjuvant and adjuvant treatment.
2. To assess the immune cell infiltrates, particularly T regulatory cells and CD4+ and CD8+ effector T cells in the resected tumors following the neoadjuvant vaccination.
3. To assess the changes in the number and function of peripheral mesothelin-specific CD8+ T cells and CD4+FoxP3+, GITR+ regulatory T (Tregs) cells for vaccine alone and vaccine with either a single dose of intravenous cyclophosphamide or metronomic cyclophosphamide following each vaccination.

**Secondary:**
1. To estimate disease-free and overall survival of surgically resected adenocarcinoma of pancreas patients treated with either the vaccine alone or the vaccine given with either cyclophosphamide administrated as a single intravenous dose or daily oral metronomic doses.
2. To estimate the effect of immune parameters on disease-free and overall survival for each treatment group.

**METHODS**

**Clinical trial:**
- A pilot, randomized three-arm, open-label, single-dose

**Patient Population:**
Our targeted accrual goal is 39 patients who are evaluable for primary immunology endpoints and who will be randomly assigned to three arms. To obtain the targeted accrual goal, it is estimated that up to 60 patients with a surgically resectable, suspected pancreatic adenocarcinoma of the head, neck, or uncinate process of the pancreas will be enrolled into this study.

**Treatment Plan and Schedule:**
All participants will receive a vaccination two week prior to undergoing the Whipple procedure, a second vaccination in 6-10 weeks following the Whipple procedure (4 weeks prior to adjuvant chemoradiation) and then vaccinations once every 28-days for a total of six vaccinations, beginning 1-2 months following completion of
chemoradiation. In Arm A, participants will receive vaccines alone. In Arm B, participants will also receive cyclophosphamide 200 mg/m² intravenously 1 day prior to receiving each vaccination. In Arm C, participants will also receive cyclophosphamide 50 mg orally and twice daily on days 1-7 for the neoadjuvant vaccination cycle (day 1 being the day of vaccination), and on days 1-7 and 15-21 for the 2nd to 6th vaccination cycles. The endpoints of the study are as follows.

**Dose:**
The vaccine consists of equal numbers (2.5 x 10⁸ each) of Panc 6.03 and Panc 10.05 cells combined into a single vaccination. The final vaccine population secretes approximately 80-100 ng/10⁶ cells/24 hours of bioactive GM-CSF. Vaccine cells from each pancreas tumor cell line, pre-irradiated and frozen at 1.25 x10⁸ cells/vial (2 vials per cell line) in an injectable formulation of Pentaspan (10% Pentastarch in 0.9% sodium chloride with 2% human serum albumin and 5% DMSO), 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. The combined 5 x10⁸ cells of Panc 6.03 and Panc 10.05 cells will be divided evenly amongst 6 syringes for intradermal injection. This preparation has been used in two completed phase II studies (Laheru et al., 2007; Laheru et al., 2008) and in two on-going phase II studies.

Cyclophosphamide (Cytoxan, CTX) is given at 50 mg twice daily (every 12 hours) per oral route or 200 mg/m² intravenously as above indicated.

**Dose-Limiting Toxicity:**
Dose-limiting toxicity (DLT) is defined as any grade 3 or 4 nonhematological 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. If the toxicity continues at ≥ grade 2 for 4 weeks then the patient will be removed from further treatment in the study.

**Safety Evaluation:**
The toxicity data collected will be descriptive, characterized according to the National Cancer Institute Common Toxicity Criteria Adverse Events (CTCAE v.3). Monitoring for adverse events will be done using an internal and external monitoring program.

**Recombinant DNA Product:**
Two allogeneic cell lines have been developed from neoplastic tissue harvested from the surgical specimens of patients undergoing pancreaticoduodenectomy at The Johns Hopkins Hospital. These cell lines have been characterized as 100% epithelial by cytokeratin staining (Jaffe et al, 1998a). In addition, these cell lines carry the same k-ras mutation as the original tumor specimen, which supports the conclusion that these lines are derived from malignant pancreatic tumor cells. The cell lines Panc 10.05 and Panc 6.03 were chosen because they contain the most common k-ras mutation at codon 12
found in greater than 90% of pancreatic cancer. Panc 10.05 and Panc 6.03 were genetically modified to secrete GM-CSF by plasmid DNA transfection (Jaffe et al, 1998a; Jaffee et al, 1998b).

**Correlative Laboratory Studies:**

Correlative laboratory studies include those to assess and compare between treatment arms the immune cell infiltrates, particularly T regulatory cells (Treg) and CD4+ and CD8+ effector T cells in the resected tumors following the neoadjuvant vaccination.

Correlative laboratory studies also include those to compare the effects of a vaccine alone versus vaccine given with either a single dose of intravenous Cy or metronomic Cy on changes in the number and function of peripheral mesothelin-specific CD8+ T cells and CD4+FoxP3+, GITR+ Tregs, and to correlate these changes with disease-free and overall survival.

**Statistical Considerations:**

In addition to safety assessment, the analyses of immune responses are primary endpoints. The study is powered to detect a minimum level of mesothelin-specific T cell responses. The test is powered for each arm separately. We do not anticipate that the sample sizes will be adequately powered to compare clinical efficacy between different arms. The analyses of the efficacy endpoints are secondary endpoints and primarily descriptive in nature and are intended to be used to generate estimates of efficacy and hypotheses to be tested in later trials.

**ANTICIPATED OUTCOME**

We anticipate that administration of pancreatic vaccine in combination with Cy is safe and feasible, as suggested by our previous studies with the same vaccine product (Jaffee et al., 2001; Laheru et al., 2008; Laheru & Jaffee et al., manuscript in preparation). Administration of preoperative vaccine is not anticipated to change the outcome of the surgery itself. We will however assess the influence of preoperative vaccination on the resectability as well as the safety of giving vaccine preoperatively. We anticipate that Cy treatment in both Arms B and C will lead to depletion of peripheral and tumor infiltrating T regulatory cells as suggested by the human study and our animal study. The study proposed here will further establish the immune modulating role of Cy by examining the more relevant, tumor-infiltrating T cell population. Finally, it will tell us which treatment module of Cy is more effective in immune modulation and should be employed in future immunotherapy studies.

**REFERENCES**