A.3.3 Technical Abstract

A Phase I Vaccine Safety and Chemotherapy Dose-Finding Trial of an Allogeneic GM-CSF secreting Breast Cancer Vaccine Given in a Specifically Timed Sequence with Immunomodulatory Doses of Cyclophosphamide and Doxorubicin

Background
Breast cancer ranks second among cancer deaths in women (American Cancer Society, 2005). In the year 2005, the American Cancer Society has estimated that 212,240 new invasive cases of breast cancer will be diagnosed, and predicts 40,410 deaths will result from breast cancer. While 80% of patients present with locoregional disease involving the breast and/or axillary lymph nodes, about half develop disseminated disease and ultimately die from it. Stage IV breast cancer is typically managed with hormonal agents or conventional cytotoxic drugs. Tumors quickly become resistant to these treatments, however, underscoring the need for novel therapeutic strategies that can be integrated with existing therapeutic modalities in an additive or synergistic fashion. Active immunotherapy with granulocyte-macrophage colony-stimulating factor-(GM-CSF)-secreting whole cell vaccines is a particularly attractive strategy for overcoming drug resistance. We propose to test the safety and bioactivity of an allogeneic GM-CSF-secreting breast cancer vaccine given in a specifically timed sequence with a range of low, immunomodulatory doses of Cyclophosphamide (CY) and Doxorubicin (DOX) in patients with Stage 4 adenocarcinoma of the breast.

Objectives

1. Evaluate the safety of the vaccination regimen by characterizing the toxicity of vaccination alone, and of vaccination combined with CY and DOX.
2. Determine the doses of chemotherapy drugs that maximize vaccine-induced immunity by measuring immune responses to HER-2/*neu* as a potential surrogate for anti-tumor immune responses. Measures of HER-2/*neu*-specific immunity will include serum antibody titers, delayed type hypersensitivity to HER-2/*neu*-derived peptides, and *in vitro* T cell responses.
3. Assess the *in vivo* immune responses induced by the vaccination regimen by vaccine site biopsies and compare these responses to those seen in our previous preclinical and clinical studies (by assessing the degree of local eosinophil, macrophage, and T-cell infiltration at the vaccine site).
4. Assess enrolled patients for time to disease progression.

Patient Population
This trial will recruit 60 patients to identify thirty eligible research subjects with Stage IV adenocarcinoma of the breast.

Study Design
This is a Phase I, three by three factorial dose-ranging study of CY and DOX with a fixed dose of breast tumor vaccine cells (5×10^8).

Treatment Plan and Schedule

The vaccination regimen is based on extensive preclinical studies that defined the timed sequencing and doses of CY and DOX with a GM-CSF-secreting vaccine that optimally induced anti-tumor immunity in the *neu* transgenic mouse. Eligible patients will receive a fixed dose of a breast tumor vaccine consisting of two irradiated allogeneic breast cancer cell lines transfected with the GM-CSF gene in a specifically timed sequence with a range of low, immunomodulatory doses of CY and DOX. Patients will receive CY intravenously on Day -1, vaccination on Day 0, and DOX intravenously on Day +7. Patients will receive three monthly vaccination cycles, with a fourth and final (boost) vaccination cycle three months from the third cycle.

Dose

The dose ranges of CY and DOX were chosen by extrapolating directly from the preclinical work. CY will be tested at 0, 250, 350, and 450 mg/m^2, and DOX will be tested al 0, 15, 25, and 35 mg/m^2.

The vaccine will be tested at a fixed dose of 5×10^8 cells based on the safety of this dose in a Phase I trial of a similar allogeneic GM-CSF-secreting vaccine for pancreatic cancer. The breast cancer vaccine consists of 2T47D-V and 3SKBR3-7 cells formulated at a ratio of two parts to one part into a single vaccination. Cryopreserved irradiated vaccine cells will be thawed on the day of vaccination, mixed in a final concentration of 4.2×10^7 cells /0.7 ml, and injected directly into the patient. Each patient will receive 12 intradermal injections, 4 in the right and left thighs and 4 in the arm opposite the side of breast surgery.

Dose-Limiting Toxicity

Dose-limiting toxicity (DLT) is defined as any grade 3 or 4 nonhematological toxicity excluding alopecia, or grades 3 or 4 hematologic 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 Terminology Criteria Version 3.0. Safety parameters to be assessed include physical examination, hematology, and serum chemistry. Monitoring for adverse events will be done using an internal and external monitoring system.

Product

Two allogeneic cell lines have been developed from the breast tumor cell lines T47D and SKBR3 that are readily available from the American Type Culture Collection. These cell lines were chosen for vaccine development because they express HER-2/*neu*, CEA, Mucin 1, and MAGE 1, all of which are known breast tumor antigens. Of these, the high level of HER2/*neu* expression by SKBR3 is particularly important since immune responses to HER-2/*neu* will be the focus of immune monitoring in this trial. T47D and SKBR3 were genetically modified to secrete GM-CSF by plasmid DNA transfection.

Summary of Current Status

To date, 15 of a planned 30 patients have been vaccinated; the first 6 patients received vaccine alone, and the remaining 9 patients have received vaccine in sequence with Cyclophosphamide (CY) and Doxorubicin (DOX). We have already completed all vaccinations on the first 11 subjects vaccinated on study. The first two cohorts received vaccine alone to characterize its safety and bioactivity; 3 individuals received 5 X 10^7 cells, and 3 individuals received 5 X 10^8 cells. Of these 6 subjects, 4 received all vaccination cycles, and 2 (cohort 2) were taken off-study early due to disease progression. Two of the four subjects who received all four vaccines continue to have stable disease while the other two showed disease progression while on long-term follow-up. The third cohort consisted of 2 subjects that received doses of CY and DOX of 250 and 15 mg /m^2 respectively. One subject completed all 4 vaccination cycles and still shows no sign of progressive disease. The other was taken off study after 3 vaccination cycles due to disease progression and died 10 months after her last vaccine due to metastatic breast cancer. The fourth cohort consisted of 3 subjects that received doses of CY and DOX of 350 and 15 mg /m^2 respectively. Two subjects completed all 4 vaccination cycles and remain disease-free while one completed 3. The fifth cohort consists of 3 subjects that will receive doses of CY and DOX of 250 and 25 mg /m^2 respectively. All 3 subjects are on study and have initiated 3 vaccination cycles. The sixth cohort consists of 2 subjects that will receive doses of CY and DOX of 250 and 35 mg /m^2 respectively. One subject is enrolled in this cohort and has initiated 2 vaccination cycles. All study subjects have experienced the expected local vaccine site reactions including erythema, pain/swelling/tenderness, and pruritis. There have been no serious adverse events related to the vaccine. The study is still open to accrual with the goal of accruing 30 research subjects.