A Feasibility Study of Combination Therapy with Trastuzumab, Cyclophosphamide, and an Allogeneic GM-CSF-secreting Breast Tumor Vaccine for the Treatment of HER-2/*neu*-Overexpressing Metastatic Breast Cancer

SCIENTIFIC ABSTRACT

Background

Breast cancer ranks second among cancer deaths in women with 40,410 deaths predicted by the American Cancer Society in 2005. While 80% of patients present with locoregional disease involving the breast and/or axillary lymph nodes, up to half develop disseminated cancer and ultimately die from it. Despite the variety of approaches and drugs available for the management of Stage IV breast cancer that is an incurable condition, there has been limited improvement in the survival of women with this disease. These limitations are thought to be related to the outgrowth of biologically heterogeneous tumor cells inherently resistant to standard therapies. It is therefore imperative to develop innovative therapeutic strategies that can be integrated with existing therapeutic modalities in an additive or synergistic fashion and that have limited toxicity as well as the potential for overcoming the problem of drug resistance in advanced breast cancer. Active immunotherapy with granulocyte-macrophage colony-stimulating factor-(GM-CSF)-secreting whole cell vaccines is a particularly attractive strategy for overcoming drug resistance. Because it is unlikely that immunotherapy alone will cure a significant number of patients with advanced solid tumors, it is important to develop strategies for augmenting the potency of both monoclonal antibodies and anti-cancer vaccines. We propose to test the safety, clinical benefit, and bioactivity of vaccine therapy in combination with Cyclophosphamide and Trastuzumab in patients with HER-2/*neu*-overexpressing Stage IV breast cancer. This study aims to test the vaccine while simultaneously abrogating the suppressive influence of $CD4^{+}CD25^{+}$ regulatory T cells (Cyclophosphamide) and augmenting antigen processing and presentation (Trastuzumab). This feasibility study is based both on observed safety to date in our current clinical trial, and on extensive preclinical modeling conducted by our breast cancer immunotherapy group. In our current trial testing the vaccine in sequence with low doses of Cyclophosphamide and Doxorubicin, six subjects received vaccine alone, and nine received vaccine in sequence with chemotherapy. There have been no vaccine-related serious adverse events. Our most recent preclinical studies suggest that combining monoclonal antibody therapy with Cyclophosphamide modulated vaccination is more effective than either alone. These studies have shown that 60% of the mice treated with a 7.16.4 (a murine equivalent of Trastuzumab), vaccination and Cyclophosphamide remain tumor-free at 50 days as compared to 10% of mice receiving vaccine and Cyclophosphamide and 30% of mice receiving 7.16.4 and vaccine. Therefore we propose to test this concept in a feasibility study of combination therapy with Trastuzumab, Cyclophosphamide, and an allogeneic GM-CSF-secreting breast tumor vaccine in subjects with HER-2/*neu*-overexpressing metastatic breast cancer.

Objectives

The primary objectives of this feasibility study are:

1. To evaluate the safety of vaccine therapy in combination with Cyclophosphamide and Trastuzumab.
2. To determine the clinical benefit of vaccination in sequence with Cyclophosphamide and Trastuzumab.

The secondary objectives of this feasibility study are:

1. To measure the magnitude of HER-2/*neu*-specific $CD4^+$ and $CD8^+$ T cell responses that develop with Cyclophosphamide and Trastuzumab sequenced with vaccination by delayed type hypersensitivity (DTH) and ELISPOT respectively;
2. To assess the impact of Trastuzumab on immune priming *in vivo* by immunohistochemistry of vaccine site biopsies at day +3 and day +7 of cycles 1 and 3, comparing cellular infiltrates to those seen in previous preclinical and clinical models;
3. To measure the impact of Cyclophosphamide pretreatment on $CD4^+CD25^+$ regulatory T cells by flow cytometry;

The developmental objectives of this feasibility study are:

1. To determine baseline and change in tumor immunohistology and gene expression profile in the context of combination vaccination therapy with Cyclophosphamide and Trastuzumab.
2. To develop the tandem tetramer/CD107a cytotoxicity assay for HER-2/*neu* specific $CD8^+$ T cells.
3. To measure novel T cell responses induced by Trastuzumab and Cyclophosphamide -modulated vaccination.

Patient Population

This study is an open label, single arm study designed to recruit up to 40 patients to identify 20 research subjects with HER-2/*neu*-overexpressing Stage IV breast cancer eligible for the intervention in on this study. The study subjects must have measurable or evaluable disease that may have been treated with any number of chemotherapy regimens for metastatic disease. Prior Trastuzumab therapy is allowed and concurrent hormone therapy and bisphosphonate therapy is allowed as appropriate.

Study Design

This is an open label, single arm feasibility study using a fixed dose of an allogeneic breast tumor vaccine (5×10^8 cells) given in sequence with immunomodulatory doses of Cyclophosphamide (300 mg/m^2) and therapeutic doses of Trastuzumab (2mg/kg) given weekly. Subject enrollment will follow a Simon two-stage design with an alpha level of 0.08 with a power of 0.86 to detect a clinical benefit of 0.45 from a null hypothesis rate of 0.20.

Treatment Plan and Schedule

Eligible research subjects will receive a fixed dose of the allogeneic 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 low dose of Cyclophosphamide and Trastuzumab. Subjects will receive 300 mg/m^2 of Cyclophosphamide on day –1, and the vaccine on day 0. Weekly Trastuzumab will be timed to coincide with Cyclophosphamide administration. Patients will receive three monthly vaccination cycles, with a fourth and final (boost) vaccination cycle three months from the third cycle.

The research subjects will be evaluated clinically and with computed tomography scans of the chest, abdomen and pelvis as well as nuclear medicine bone scans for evidence of disease progression as clinically indicated or by the protocol. In addition, they will be monitored for elevation of the breast tumor markers CEA and CA15-3 if known to be associated with their disease. These studies are sensitive for detecting early new metastases. Cardiac function will be evaluated by MUGA or echocardiograms and acute toxicities will be assessed from the results of hematological profiles and blood chemistries.

Dose

The dose of Cyclophosphamide will be 300mg/m^2 administered intravenously. The dose of Trastuzumab will be 2mg/kg/week. The initial dose for subjects initiating Trastuzumab therapy on this study will be 4 mg/kg. The breast cancer vaccine will at a fixed dose of 5 X 10^8 cells and 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 x 10^7 cells /0.7 ml, and injected directly into the research subject. Each subject 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 for Adverse Events version 3.0 (CTCAEv3.0). 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. T47D and SKBR3 were genetically modified to secrete GM-CSF by plasmid DNA transfection.