

**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.**

NON-TECHNICAL ABSTRACT

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. Immunotherapy is a particularly attractive strategy for overcoming drug resistance and can be integrated with existing therapeutic modalities in an additive or synergistic fashion. Immunotherapy is a type of treatment for cancer based on the idea that the immune system can be activated to destroy cancer cells that might be resistant to hormonal therapy and chemotherapy. A vaccine is a kind of immunotherapy that delivers an antigen (something that activates the immune system) so that the immune system recognizes cells with that antigen as foreign and destroys any cells that display that antigen.

The allogeneic breast tumor cell vaccine consists of two types of breast tumor cells developed from the tumor cells of patients with breast cancer. The human granulocyte-macrophage colony-stimulating factor (GM-CSF) gene was used to genetically modify the breast tumor cells to secrete GM-CSF. GM-CSF is a substance made by the body that helps the immune system recognize a tumor and destroy it. The vaccine cells were irradiated to prevent them from growing or dividing. The cells themselves are not radioactive. The cells are stored frozen until the day of vaccination. The total number of cells in each vaccine will be 500,000,000, divided into twelve injections given in the thighs and arms. The choice of twelve injections for each vaccine is based on the volume of the vaccine and a finding that the body has a better chance to respond to the vaccine if it is injected into a number of different areas.

We propose to test the safety and bioactivity of an allogeneic GM-CSF-secreting breast cancer vaccine when given in a specifically timed sequence with Cyclophosphamide and Trastuzumab, two drugs commonly used to treat breast cancer. In this study the Cyclophosphamide is used at lower doses than usual to help the vaccine to activate the patient's immune system. Trastuzumab will be given at doses that are commonly used to treat breast cancer and it may also increase the immune response. The dose and scheduling of Cyclophosphamide and Trastuzumab used are based on testing the drugs with a GM-CSF-secreting vaccine in mice that get breast cancer, and are the ones that enabled the vaccine to induce the most potent anti-tumor immunity. The dose of vaccine cells is based on the safety of the same dose of a similar GM-CSF-secreting vaccine for pancreatic cancer. This breast cancer vaccine has also been given to people with breast cancer by itself, and with Cyclophosphamide and another chemotherapy drug,

New Protocol Submission May 12, 2006

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Doxorubicin. To date, people have had no vaccine-related serious side effects, but not enough people have received the vaccine to know if it treats breast cancer. The vaccine is experimental and has not been approved by the U.S. Food and Drug Administration (FDA). However, the FDA has permitted its use in this research study. This is the second study of this breast cancer vaccine and the first study to test this vaccine with Trastuzumab.

The main purposes of this study are 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 will also to test whether the Cyclophosphamide can eliminate the suppressive influence of regulatory T cells, and whether Trastuzumab can increase antigen processing and presentation. These drug activities may make the immune system react better and enhance the effects of the vaccine in treating breast cancer.

The study is open to men and women with HER-2/*neu*-overexpressing metastatic breast cancer. Concurrent hormone therapy and/or bisphosphonates (standard breast cancer therapy that is not chemotherapy or other investigational therapy) is allowed. Patients may have received Trastuzumab in the past or continue on it while participating in this study. About 40 people with HER-2/*neu* positive breast cancer will enter in the study. About 20 will pass the screening tests and receive the vaccine.

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. Patients will receive 300 mg/m² of Cyclophosphamide on day -1, and the vaccine on day 0. Weekly Trastuzumab will be timed to coincide with Cyclophosphamide administration. Research subjects will receive three monthly vaccination cycles, with a fourth and final (boost) vaccination cycle three months from the third cycle.

Blood samples to measure GM-CSF levels will be taken on the day of vaccination, every day for 4 days, and then on day 7 after vaccination. Blood samples to evaluate the safety of the vaccinations will be taken about once a week for one month following each vaccination. During studies of the breast vaccine and similar vaccines in renal cell cancer, prostate cancer, pancreatic cancer, and non-small cell lung cancer, local symptoms of swelling and redness developed at the vaccine site between 2 and 7 days after vaccination. In this study, if the subject's vaccination site shows swelling over 1 cm in diameter, a skin biopsy will be taken. The skin biopsy will be evaluated to determine to what types of cells are important to the immune response. Based on our previous preclinical and clinical data, the biopsy will be taken on day 3, and possibly on day 7, after the first and third vaccinations. Other tests and evaluations include history and physical examination, vital signs, CT of the chest, abdomen, and pelvis, nuclear medicine bone scan, pre-vaccination biopsy, blood for immune monitoring, and a skin test for delayed-type hypersensitivity (DTH) that is like a PPD test and involves injecting pieces of a protein antigen (HER-2/*neu*) that is delivered by the breast cancer vaccine. The

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purpose of the DTH test is to evaluate whether the research subject has developed a systemic immune response to the breast cancer vaccine. Tumor core needle biopsies will be obtained at baseline, and on days 0 and +14 of vaccine cycle 1 only.

Patients will be evaluated clinically and with laboratory testing for evidence of disease progression after each cycle or when otherwise clinically indicated. Computed tomography (CT scan) of the chest, abdomen, and pelvis and nuclear medicine bone scan will also be performed to evaluate disease status prior to starting the study, after vaccine cycle 3 and prior to and after vaccine cycle 4. About every three months cardiac function will be evaluated.