The Prognostic Significance of Her2/*neu* Protein Over-Expression in Patients With High-Risk Primary Breast Cancer That Have Undergone High-Dose Chemotherapy With Stem Cell Support

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A. Study Purpose

To determine the prognostic significance of Her2/*neu* (c-erbB-2) protein over-expression in patients with high-risk stage II (10 or greater lymph nodes) and stage III breast cancer that have undergone high-dose chemotherapy with stem cell support.

B. Rationale

Breast cancer is the most common malignancy encountered by women and is the second-leading oncologic cause of mortality in women after lung cancer. Currently, patients with breast cancer and greater than four lymph nodes involved with malignancy have a 26% overall survival rate after 10 years.[1] In an effort to understand why some tumors are more resistant to therapy than others, a number of pathological variables have been examined. Her2/*neu* is a gene encoding a transmembrane tyrosine kinase receptor similar in structure to epidermal growth factor receptor. Over-expression of this receptor is known to reflect increased proliferation of a breast cancer cell[2], and its presence has been proven to be an independent, negative prognostic factor in many breast cancer populations. 15-30% of breast cancers over-express this protein.[3]

High-dose chemotherapy for breast cancer came to widespread attention after a number of promising observations in the lab and then subsequently through small phase I and II trials. The regimens vary but ordinarily involve 10-fold higher doses of conventional chemotherapy based upon cyclophosphamide, doxorubicin, 5-fluorouracil, and carmustine.[4] Extensive controversy exists surrounding the long-term benefits of high-dose therapy when weighed against the risks of its toxicity. Prognostic variables such as Her2/*neu* over-expression have been studied in the population of patients with metastatic disease and have yielded conflicting results regarding the test's accuracy as a predictor of survival.[5] For patients considering high-dose chemotherapy that have high-risk primary breast cancer, defined as stage II disease with ten or greater lymph nodes positive for metastatic disease or patients with stage III disease, reliable prognostic variables may aid in making treatment decisions.

To explore this, a pilot study from Park Ridge, Illinois, of 25 patients examined the prognostic significance of Her2/*neu* over-expression in women with high-risk primary breast cancer that underwent high dose chemotherapy with autologous stem cell support.[6] With a three-year median follow-up, 4 out of 4 women whose tumors over-expressed Her2/*neu* relapsed after 6 to 18 months; in comparison, 0 out 21 women with tumors expressing low to normal levels of Her2/*neu* protein relapsed. This finding has not been confirmed with a larger number of subjects. Currently, Columbia's transplant program follows approximately 160 patients who underwent high-dose chemotherapy with autologous stem cell support for high-risk primary breast cancer. The proposed study will examine these patients' Her2/*neu* status and its relation to clinical outcome over a median follow-up of three years. If this study confirms the results of the pilot study, then patients with this stage of disease and whose tumors over-express Her2/*neu* protein may be advised to decline high-dose chemotherapy or to enter a protocol that offers high-dose chemotherapy and anti-Her2/*neu* immunotherapy.

C. Study Design and Statistical Analysis

This is a retrospective study examining the prognostic significance of Her2/*neu* protein over-expression in patients with advanced breast cancer that have undergone high-dose chemotherapy with stem cell support. Each of the patients in this study underwent chemotherapy according to the CAMP 001 protocol (also known as the CAMP 100 protocol for administrative purposes only) or to the CAMP 014 protocol. These protocols utilized virtually identical regimens of high-dose chemotherapy with autologous stem cell support to treat patients with high-risk primary breast cancer. Patients in the CAMP 014 protocol were additionally randomized to receive one of two non-specific immunotherapy regimens after treatment. No difference in clinical outcome has been found between the patients from the CAMP 001/100 protocol and the patients in either arm of the CAMP 014 protocol.[7]

Representative specimens of each patient's original tumor will be requested by fax and sent to the Department of Pathology. A copy of the pages in each patient's protocol consent form relevant to tissue release will be included in each request. The amount of Her2/*neu* protein will be quantified by an immunohistochemical method using the Dako polyclonal antibody kit.[8] Patient outcomes will be obtained from the transplant program physicians and referring physicians. The pathologist will be blinded with regard to patient outcomes.

The patients will be stratified into three groups, according to their respective protocol and study arm. The primary endpoints will be time to relapse and time to death. These data will be summarized in Kaplan-Meier survival curves. Secondary endpoints, to be evaluated by Cox proportional hazards modeling, will be the prognostic significance of age, stage of disease, ethnicity, and the following pathological parameters: nuclear grade, evidence of vascular invasion, estrogen- and progesterone-receptor status, and % of tumor cells in S phase. For a statistical power of 80%, 35 patients overall with tumors over-expressing Her2/*neu* will be needed if one estimates the relapse rate at three years to be 60% in the group over-expressing Her2/*neu* and to be 20% in the low-to-normal Her2/*neu* expression group.

D. Study Procedures

As this is a retrospective study evaluating available pathologic specimens, no procedures will be conducted.

E. Study Drugs

No drugs are being administered as part of this study.

F. Medical Devices

No medical devices are being used in this study.

G. Study Questionnaires

No questionnaires will be used in this study.

H. Study Subjects

All subjects enrolled in this study fulfilled the eligibility requirements for the CAMP 001/100 or CAMP 014 protocol. Patients with metastatic disease upon enrolling in the CAMP 001/100 protocol were excluded from this study. The eligibility requirements were as follows:

- Breast cancer, histologically confirmed.
- -Stage II (with ten or greater lymph nodes involved with malignancy).
- -Stage III (any T, N2 or N3, M0)

- Ineligible for another high-priority national or institutional study.
- Completion of at least three cycles of chemotherapy:
- -Stage II: 4 to 6 courses of doxorubicin- and/or taxol-based adjuvant chemotherapy.
- -Stage III: Complete response or partial response to 4 to 6 courses of a doxorubicin- and/or taxol-based regimen.
- Beta-HCG test negative (unnecessary if the patient is post-menopausal by LH and
- FSH levels).
- Brain CT or MRI without visible metastases.
- MUGA scan shows an LVEF greater than or equal to 45%.
- ECOG performance status of 0 or 1.
- White blood cell count >3,000/ul and platelet count >100,000/ul (CAMP 001/100 only).
- Age between 18 and physiologic 60 years (CAMP 001/100 only).
- HIV test negative.
- Creatinine <1.5x normal.
- Bilirubin <2x normal.
- SGOT <1.5x normal (CAMP 001/100 only).
- Bilateral bone marrow biopsies and aspirates performed.
- Blood tests prior to study: CBC with differential, CMV and HSV antibodies, reticulocyte count (CAMP 001/100 only); CAMP 014 only: SMAC, LFT's, electrolytes.
- CT of chest, abdomen, and pelvis (CAMP 014 only).
- Ability to harvest >$1.0x10^6$ CD34+ cells/kg and/or $4x10^8$ MNC/kg (CAMP 014 only).

- The CAMP 001/100 protocol high-dose chemotherapy regimen:

- Cyclophosphamide 1500 mg/m^2/day iv x 5 d until day -3
- Thiotepa 125 mg/m^2/day iv x 4 d until day –4
- Carboplatin 200 mg/m^2/day iv x 4 d until day –4
- Mesna 1875 mg/m^2/day iv x 6 d until day -2

- Marrow and/or peripheral stem cell infusion on day 0
- Post-consolidation mastectomy and radiotherapy if appropriate
- Tamoxifen 20mg po daily for five years

- The CAMP 014 chemotherapy protocol differs with the above regimen only in that thiotepa and carboplatin are administered in the same dose for the same number of days, ending on day –3 instead of day –4.
- In addition, CAMP 014 participants were randomized at study entry to receive either of the following two immunotherapy regimens after chemotherapy:

A: Cyclosporin A 1.25 mg/kg/day iv over 4 hr bid, or 3.75 mg/kg/day iv divided into 2 doses bid; day 0 until discharge

Interferon gamma 0.025 mg/m^2 sc qod; day 7 until day 28

B. Interleukin-2 1.0 x 10^6 U/m^2/day sc qd; 28 days, beginning after ANC > 0.5 x 10^9 and platelet count > 20,000

I. Recruitment of Subjects

There will be no recruitment of patients for this study. The only patients whose tumors are being studied are those who completed the CAMP 001/100 or CAMP 014 protocol.

J. Confidentiality of Study Data

Any information obtained during this study and identified with the patient will remain confidential. All subjects will be given a unique identifier; this will be used for all further evaluations. All the data will be stored on a mainframe network computer. The data will only be accessible to members of the research team through personalized logon codes and to the physicians caring for the patients.

K. Potential Conflicts of Interest

None.

L. Location of the Study

Her2/*neu* staining will be done in the Department of Pathology.

M. Potential Risks

None.

N. Potential Benefits

Subjects may or may not benefit personally from this study if their Her2/*neu* status was previously unknown and they subsequently become eligible for Herceptin. Herceptin, a monoclonal antibody directed against the Her2/*neu* protein, has proven efficacy in some patients with stage IV whose tumors over-express the Her2/*neu* protein.[9]

O. Alternative Therapies

No therapy is involved in this study.

P. Costs and Compensation to Subjects

The subjects play no role in this pathology study. There will be no additional costs to the subjects and no financial compensation.

Q. Minors as Research Subjects

There are not any minors in this study.

R. Radiation or Radioactive Substances

This study will not involve the use of radiation or radioactive substances.

S. Citations

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