Predictive Value of Her-2/Neu over-expression and PTEN deletion in High-Risk Primary Breast Cancer Patients Treated with High-Dose Chemotherapy and Stem-Cell Support.

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A. Study Purpose

Evaluate for a prognostic significance of Her-2/Neu expression and PTEN deletion in patients with highrisk primary (Stage II with 10 plus lymph nodes and Stage III) breast cancer who have undergone treatment with high-dose chemotherapy and stem-cell support.

B. Rational

Breast cancer is the leading malignancy among women in the United States with a 12% lifetime accumulative risk of developing disease[1]. It is estimated that in the United States breast cancer caused 47,000 oncologic deaths in 1999[2]. While screening and adjunctive chemotherapy has resulted in the diagnosis of more early-stage disease and in improved survival, mortality among those presenting with advanced disease remains high. Ten year mortality among patients presenting with a large tumor, 10 or more lymph nodes positive, or inflammatory disease is 85%, and mortality is 95% among the 20,000 patients a year presenting with metastatic disease[2] .

Numerous attempts have been made to improve on the response rate to therapy and survival of patients with advanced breast cancer. One avenue of investigation has been treatment of high-dose chemotherapy 'with autologous stem cell support. High dose protocols generally involve chemotherapy doses 10 fold higher then is given in conventional chemotherapy. This will ablate the patient's blood forming cells, which are then replenished with the patients own, previously harvested, blood stem cells. High dose therapy has been controversial because the increased toxicity of the therapy must be compensated for by a decrease in disease progression and mortality. Results of pre-clinical and phase H trials were promissing2, but improvements in disease free survival and overall survival have yet to materialize in randomized phase III trials. Notably, the Philadelphia trial of 553 stage IV patients randomized to high-dose vs. standard chemotherapy after complete or partial response to convention chemotherapy showed no survival benefit with high dose chemotherapy at three years[4]. Preliminary results for a similar trial in high-risk stage II and stage III patients has also shown no significant difference in disease free or overall survival[5].

One barrier to evaluating the effectiveness of novel treatments for advanced breast cancer is the high degree of variability in disease course and response to chemotherapy. Standard chemotherapy with 5-FU, doxyrubicin, cyclophosphemide, and carmustine produces no or only partial response in 85% of patients, yet 15% achieve complete response and 3% are disease free after 10 years[2,3]. Convention methods of tumor imaging and pathologic evaluation have not been able to predict tumor behavior and response to chemotherapy.

While there has been an increasing understanding of the molecular events leading to cancer, translating this into practical molecular markers that can be used to predict tumor behavior has been dificult[6]. Through Columbia's transplant program we have assembled a database of approximately 100 patients who have undergone high-dose chemotherapy with autologous stem cell support. The proposed study will evaluate these patients for two molecular markers which may help us gain insight into their

clinical behavior and delineate the sub-groups of patients who may or may not benefit from high-dose chemotherapy in the future.

a. **Her-2/*Neu***

Her-2/Neu is a gene encoding a transmembrane tryosine kinase receptor with a structure similar to the epidermal growth factor receptors; it is overexpresed in approximately 30% to 45% of all breast cancers.[7]

Stationed on the cell surface Her-2/Neu dose not interact with other proteins in the cell unless it becomes activated. Activation occurs when Her-2/Neu forms homodiamers or heterodiamers with other members of the epidermal growth factor receptor family. Diamer formation and activation can occur through binding to a ligand, mutations in the transmembrane region of the protein, or from overexpresiton .[8] Once active Her2/*Neu* interacts with cellular proteins which have a role in the signal transduction pathway and cell proliferation. Experimental models have shown that Her-2/Neu can increase cancer cell metastatic potential and invasive ability.[8]

There have been several large studies showing that Her-2/Neu over-expression is associated with decreased disease free survival in node positive early stage breast cancer.[9] In 1996 Bitran JD et al. studied Her-2/Neu in 24 high risk primary breast cancer patients (<10 LN) who underwent high-dose chemotherapy with stem cell support. Four out of 4 patients with Her-2/Neu over-expression relapsed in 6 to 18 months and 0 out of 21 Her-2/Neu negative patients relapsed with 36 month follow up[13] . A subsequent study of 146 high-risk stage 11 and stage IH cancers showed Her-2/Neu overexpression in 45% of cases. The Her-2/Neu positive patients had a greater likelihood of decease progression, 55% vs. 15%, and a decreased overall survival, 60% vs. 85% at 48 months

b. PTEN

PTEN is a candidate tumor suppressor gene that was mapped to chromosome 10q23.3 in 1997. The PTEN protein has two domains. One domain is a tryosine phosphetase that can dephosphorylate many proteins involved in the cell signaling pathway and the second similar to tensin, protein that interacts with actin filaments and cell adhesions [14] . Deletions in PTEN have been found in many metastatic solid tumors including glioblastomas, bladder tumors and prostate cancers. Germline PTEN deletions have also been shown in Cowden's syndrome. an autosomal dominant disorder associated with among other things a 50% chance of developing breast cancer [14] In breast cancer cells normal PTEN expression has been shown to decrease ligand-induced signaling through the P13K cascade and in turn decrease cell growth and increase cell apoptosis[15]. Studies have shown mono-allele deletions in 30-45% of invasive breast cancers, and PTEN deletions has been associated with a poor histologic phenotype, invasive disease, and a negative ER/PR Status[14,16].

While single allele deletions have been found in a high proportion of tumors, only approximately 5% of cancers have had mutations in the second PTEN allele, thus bringing to question if these cancer cells truly have loss of PTEN function[17]. Perren A, et al. published a study in 1999 where inummohistochernical stains were used to determine the absence of PTEN protein in ductal adnocarcinomas. Perren looked at 33 cancers using a monoclonal antibody (6h.2.1). The antibody specificity was tested using Western Blot Analysis on known PTEN positive and negative cell lines, and adjacent normal maxillary epithelial cells provided in vitro controls for defining positive and negative staining. Eleven of 33 or 33% of cancer 6ells either lacked or underexpresed PTEN. All of these tumors were also found to have mono-allele but not bi-allele deletion of 10q23. This study raises the question of if in fact PTEN mono-allele deletion with decrease protein expression plays a role in breast cancer development and whether loss of the PTEN protein predicts a more aggressive or less adhesive tumor.

C. Study Design And Statistical Analysis

For each patient, data regarding survival and disease progression will be collected as of l/l/00 to 7/l/00. Every six months each patient is screened for disease progression with chest, abdomen and pelvic

CT scans, a bone scan and liver function tests. Disease free survival will be defined as time from stem cell transplant to time of first evidence of metastases. Survival will be defined as time from stem cell transplant to death.

A representative sample of each patient's tumor will be collected and immuno-blank slides will be produced. For patients with tumor resected at outside hospitals a formal request and a copy of the patient's consent form will be sent by fax to the appropriate Department of Pathology. The outside hospital pathologist will determine the representative tumor block from the patient's resection. For patients with tumor resection at CPMC immuno-blank slides will be produced from the tumor block that was used for ER/PR status. If this block is not available a pathologist will review the case and select an appropriate block.

Her-2/Neu and PTEN will be determined using an inummohistochernical (IHC) method. Pathologist will be blinded to patient outcome. There are currently two frequently used methods to evaluate Her-2/Neu in paraffin fixed specimens: florescent in situ hybridization (FISH) and IHC. Traditionally IHC has been a more variable method of determining Her-2/Neu status. There are more then 30 commercially available antibodies each with different specificities and sensitivities; both would vary from 50% to 90% base on FISH as the gold standard. The Dako Corporation produces an IHC kit with a rabbit CB-11 antibody and controls. Her-2/Neu is measured as present or absent +0 vs. +1-3, and then quantified based on the percentage of cells positive. A recent comparison showed a 91 % concordance between FISH and IHC using the Dako method[5]. Her-2/Neu will be quantified using an internal protocol using the Ab-3 Rabbit monoclonal antibody (Oncogene Inc.) that has been shown to be in concordance with the Dako method for determining absence or strong over-expression of Her-2/Neu (+0 or +3). For patients with slight to moderate over-expression (+1 to +2), slides will be re-stained using the Dako monoclonal antibody kit[11]. Tumors positive for Her-2/Neu will be sent to Yale University School of Medicine Department of Pathology and re-stained using the PN2A antibody selective for the phosphorylated Her-2/Neu with methods previously described by DiGiovanna [12] . PTEN has been less well characterized, will be stained using the 6H2.1 monoclonal antibody as described by Perren et al.

The primary endpoints will be disease free survival and overall survival. The patents will be stratified by disease stage. Univariant analysis of Her-2/Neu and PTEN both as dichotomous variables with disease free survival and overall survival will be performed using a two-sided paired t-test. Data will be summarized on Kaplan-Meier survival curves. If the estimated relapse rate is 55% for Her-2/Neu positive patients and 15% for Her-2/Neu negative patients, 35 overexpressors will be needed for a statistical power of 80%. For PTEN the primary end points will be documentation of the incidence, and effect on overall survival. Expecting an incidence of 30 - 45% of PTTEN under-expression the study will be powered to detect a 30% difference in the proportion of patients progressing. Multivariate analysis will be done with Cox proportional hazards analysis. Factors analyzed will include disease stage, ER/PR status, poor histologic grade and evidence of vascular invasion, % of tumors in S-phase.

D. Study Procedures

None

E. Study Drugs

None

F. Medical Devices

None

G. Study Questionnaires

None

H. Study Subjects

All patients have met the eligibility requirements for the CANT 001/100 and CANT 014 protocol. These eligibility requirements include the following:

- Histologically confirmed breast cancer, Stage 11 and with 10 or more involved lymph nodes, Stage III, Stage IV
- Age between 18 and 60 years old
- neligible for another high-priority national or institutional study
- Completion of at lest three cycles of doxorubicin and/or taxol based conventional chemotherapy. For Stage II, 4-6 coursed, Stage III and Stage IV, CR or PR to 4-6 courses.
- Beta-HCG negative or postmenopausal by LH and FSH
- Brain CT or N4RI without visible metasticies.
- LVEF 45% or greater on MUGA
- ECOG performance 0 or I
- WBC >3,000/ul, Platelet >100,000/ul (CANIP 001/100 only); Creatinine <1.5 x normal and Bilirubin <2 normal (CANW 00 1/100, 0 14)
- HIV negative.
- Ability to harvest >1.OX106 CD34+ cells/kg and/or 4xIO8 MNC/kg (CAW 014 only)
- Stage IV patients will be excluded from this study
- All patient in the study received the following chemotherapy regimen (Day 0 day of transplant).
- CAMP 001/100:
    - Cyclophosphamide 1500 mg/m2/day IV x 5 days until day -3
    - Thiotepa 125 mg1m2/day IV x 4 days until day -4
    - Carboplatin 200 mg/m2/day IV x 4 days until day -4
    - Mensa 1875 mg/m2/day IV x 6 days until day -2
    - Peripheral stem cells or marrow at day 0
    - Post consolidation radiotherapy or mastectomy if necessary
    - Tomoxifen 20 mg po qd for five years

CAMP 014 patients received Thiotepa and Carboplatin for 4 days but ending day -3. Patients were randomized to either

1.) Cyclosporin A 1.25 or 3.75 mg/kg/day IV bid day 0 until discharge and INFy 0.025 mg/m2 sc qod day 7 to day 28, or
2.) Interleukin-2 1.0 x 10^6 U/m2/day sc qd for 28 days, beginning after ANC > 500 x10^7 and Platelet count > 20,000.

I. Recruitment Of Subjects

CAMP 001/100, CAMP 014 patients, and no additional recruitment

J. Confidentlality Of Data

All subjects will have a unique identifying number for the purposes of this study. Data will be kept on desktop computers accessible only to members of the research team and transplant teams. All information will be confidential.

K. Potential Conflict Of Interest

None.

L. Location Of Study

Data stored at CPMC, Department of Hematology and Oncology and pathology studies will be done at the Columbia and Yale Departments of Pathology.

M. Potential Risks

None

N. Potential Benefits

Patients will have access to their Her-2/Neu status and PTEN status upon request.

O. Altenative Therapies

None

P. Compensation Of Subjects

None

Q. Costs To Subjects

None

R. Minors As Research Subjects

None

S. Radiation Or Radioactive Substances

None

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