Prognostic relevance of micrometastatic breast cancer cells in the bone marrow

Jennifer J. Wheler

A. Study Purpose and Rationale

Breast carcinoma is the most common malignant disorder in women, accounting for approximately 185,000 new cases each year in the United States. Current staging modalities provide good prognostic estimates; however, they do not provide an unequivocal means of predicting outcomes, particularly in intermediate and advanced stages. Recent experimental studies have suggested that bone marrow micrometastases are a strong and independent prognostic indicator in patients with stage Il or stage III disease, and one which may be used as a marker to identify early stage breast cancer patients with a poor clinical outcome. We propose to further evaluate the prognostic significance of bone marrow micrometastases in these patients. In addition, this study will serve as an initial evaluation of the possible role of bone marrow micrometastases as a predictor of the response to IL-2 immunotherapy.

B. Study Design and Statistical Analysis:

This is a retrospective study to be performed by a chart review of 120 patients with stage 11 or III breast cancer who were treated at Columbia-Presbyterian Medical Center between 1996 and 2000. These patients were each enrolled in one of three separate study protocols that examined response to high dose chemotherapy with autologous bone marrow transplantation. These protocols were previously approved by the IRB.

The study population will be divided into two groups based on the presence or absence of micrometastases in the bone marrow. All patients received high dose chemotherapy and autologous bone marrow transplantation. In addition, 20 of these patients were randomized to receive IL-2 immunotherapy. This randomization was performed without regard to bone marrow micrometastatic disease. There was no crossover of patients between treatment groups.

Endpoints for the study include disease recurrence and death. Patient survival over time will be evaluated using Kaplan-Meier life table curves. To compare differences in survival between groups, the log-rank test will be employed. The predictive value of micrometastases will be compared to that of other variables including: lymph node status; tumor size; estrogen receptor status; histology; her-2-neu. status; menstrual status, and race. The prognostic information obtained from bone marrow micrometastases with that provided by these other factors will be determined using the Cox proportional hazards analysis.

The size of the study population was based on a power analysis assuming that approximately 36% of the study population would have micrometastases, and assuming that disease free survival at two years would be approximately 70% for those subjects with micrometastases and 95% for those subjects without micrometastases. In order to have a power of greater than 80%, at least one hundred subjects are required. The number of patients who received IL-2 may not be great enough to determine whether bone marrow mierometastases are a predictor of response to immunotherapy. However, if such an effect is apparent the data will be analyzed by the same statistical methods described above.

C. Study Procedures

Diagnosis and staging of breast cancer was performed in accordance with the guidelines laid out in the previous study, which are essentially the same as those employed in standard clinical practice. Patients received: breast biopsy/lumpectomy/mastectomy with pathological tissue analysis and lymph node dissection; chest x-ray; full body CTs including head, chest, abdomen and pelvis, and bone scan. In

addition to these standard tests patients also underwent a full panel of serological testing including HIV and hepatitis panel. Subsets of the patient population received pathologic testing of breast tissue for estrogen and progesterone receptors and her-2-neu status.

Bone marrow harvesting with concomitant bone marrow biopsy was performed before chemotherapy was initiated. Patients then underwent induction chemotherapy outside of the trial under the direction of their regular oncologists. After one to two cycles of induction chemotherapy, peripheral blood stem cells were harvested via a central venous catheter. Following completion of this standard chemotherapy regimen, patients received a 96-hour continuous administration of high dose chemotherapy with cyclophosphamide, thiotepa and carboplatin via central venous catheter. On the third day after completion of high-dose chemotherapy infusion, bone marrow and peripheral stem cells were reinfased. G-CSF was injected subcutaneously daily beginning on the day prior to marrow reinfusion and concluding upon the recovery of a normal white blood cell count.

Patients have been and will continue to be followed clinically for a period of at least two years post-treatment and will undergo standard diagnostic procedures for extent-ofdiseage evaluation.

Pathological analysis of bone marrow biopsies is central to this study. After accrual of biopsies, slides will be prepared by using thin cuts from the bone marrow blocks. Slides will then be stained with three separate monoclonal antibodies: ae1, ae3, k11. These stains comprise 3 broad spectrum antibodies that detect most high and low molecular weight cytokeratin epitopes. Cytokeritin epitopes are not specific for epithelial cells and are found on other cell lines. Therefore, following the staining of the slides, the pathologist will examine each slide under the microscope. Since epithelial cells are not normally found in the bone marrow, their presence may be interpreted as a metastatic cell.

D. Study Subjects:

Study subjects are drawn from those subjects who participated in one of three protocols involving high dose chemotherapy regimens and autologous bone marrow transplantation. These subjects are comprised of women with either high risk breast cancer (10 or more lymph nodes positive for malignancy); locally advanced breast cancer (stage III) or breast cancer that has recurred after treatment or has spread (metastasized) to distant sites. Of these subjects, only those with stage II and III are to be included in the evaluation of micrometastases in the bone marrow.

E. Recruitment of subjects:

Subjects were recruited into the prior high dose chemotherapeutic trials through their own general practitioners and oncologists and through a referral system of physicians.

For our study, patients were drawn from these existing high-dose chemotherapy protocols.

F. Confidentiality of Study Data:

Prior to participation in the high dose chemotherapy trial, patients signed a consent form that had a confidentiality clause stating that information obtained in this study is to be kept confidential and that at no time would a patient be identified without first signing a release.

G. Potential Risks:

Patients will not undergo any further risks by participating in the proposed study of the evaluation of bone marrow micrometastases. All potential risk to the patient was related to the prior studies in which patients underwent high dose chemotherapy and autologous bone marrow transplantation. Those risks included the side effect profile of high dose chemotherapeutic agents: bne marrow suppression; heart failure; lung damage; liver damage; kidney damage; bladder irritation; skin changes; hair loss; menstrual irregularity or cessation; infertility; allergic reaction; nausea, vomiting and anorexia

H. Potential Benefits

The average survival after the first diagnosis of metastatic breast cancer is about two years despite treatment with conventional dose chemotherapy regimens. Patients with high risk stage II breast cancer have more than a 60% chance of developing metastatic disease within 5 years of diagnosis. Patients with bulky and/or inflamed primary breast cancer (stage III) have a greater than 80% chance of developing metastatic breast cancer within 2-5 years of the original diagnosis. The possible benefit for the patients of participating in this study was that if the treatment plan is effective, they may have a longer disease-free period than that obtained with conventional dose chemotherapy.

The benefit to the subject of participating in the study is not readily measurable in terms of a participant's own given outcome. In allowing their bone marrow to be used in this study, each subject is making a contribution to the future treatment of breast cancer patients. It is unlikely that a subject would benefit directly from their contribution.

I. Alternative Therapies:

Other possible therapeutic options for subjects who received the high dose chemotherapy and stem cell transplantation include treatment with conventional doses of combination chemotherapy; new experimental chemotherapeutic agents or no therapy or supportive care without chemotherapy.

J. Compensation to subjects:

The subjects will not receive financial compensation for their participation in this study.

K. Cost to subjects

Participation in the previous studies required that a subject be able to either have their treatment covered by an insurance company or the ability to pay out-of-pocket. For the current proposed study, there would be no further cost for the patient.