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A Comparative Study of High-Dose Chemotherapy with Autologous Hematopoietic Progenitor Cell Transplantation versus Conventional Chemotherapy As Treatment for Metastatic Breast Cancer

By

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Thesis submitted to the School of Graduate Studies and Research

in partial fulfillment of the requirements for the M.Sc. degree in Epidemiology

University of Ottawa

November, 1998

Abstract

Objectives: Data from one small randomized trial has suggested a benefit for high-dose chemotherapy/autologous hematopoietic progenitor cell transplantation (HDCT/AHPCT) as compared with conventional chemotherapy (CCT) in patients with metastatic breast cancer. However, the study was considered to have had some limitations based on methodology, analysis, and sample size. The present study sought to compare differences in outcome in patients with metastatic breast cancer undergoing HDCT/AHPCT as compared with historical controls undergoing CCT. The principal endpoints analyzed were overall survival and time to first failure after beginning chemotherapy. Secondary outcomes included an analysis of predictors of time to first recurrence of breast cancer, analyses of prognostic factors for overall survival at recurrence, of time to first failure from the time of beginning chemotherapy, and for survival after HDCT/AHPCT.

Patients and Methods: The experimental group consisted of data from 154 patients receiving HDCT/AHPCT between the years 1991-1995 from two transplant centres (University of Nebraska Medical Center, Northeastern Ontario Regional Cancer Centre). Selection criteria similar to those used to select patients for HDCT/AHPCT were used to define an appropriate historical control group from the records of patients treated at the Ottawa Regional Cancer Centre over the same time period. From the records of 235 potentially eligible patients, 135 controls were selected. Univariate and multivariate time to event analyses (overall survival and time to failure) were performed and the outcomes compared between the two groups.

Results: The median overall survival of all patients after the development of metastatic disease was 27.4 months (95% confidence interval [CI]=25.6-33.1 months). The median overall survival for patients who received CCT was 25.6 months, and for patients who received HDCT/AHPCT was 28.1 months (P =0.39 by logrank). The median time to failure for all patients after beginning chemotherapy was 12.4 months (95% CI = 11.2-14.3 months). The median time to failure for patients who received CCT was 9.8 months, and for patients who received HDCT/AHPCT was 15.6 months (P =0.005 by logrank). The use of multivariate analysis to adjust for baseline and therapy related prognostic differences between groups revealed a statistically significant difference in favor of HDCT/AHPCT for both overall survival (Hazard Ratio [HR]=0.62, 95% CI=0.27-0.97, P=0.008) and time to failure (HR= 0.54, 95% CI = 0.24-0.84, P<0.001). The median duration of survival after HDCT/AHPCT was 16.5 months (95% CI=13.7-21.7 months), and was not different for patients treated at the University of Nebraska Medical Center (15.3 months, 95% CI=12.2-23.5 months) when compared with patients treated at the Northeastern Ontario Regional Cancer Center (16.3 months, 95% CI=13.8-25.6 months) in univariate (P =0.70) or multivariate (P=0.65) analysis.

For all patients, independent predictors of time to initial recurrence after diagnosis included advancing initial clinical stage (HR=1.58, 95% CI=1.47-1.69) and a borderline effect of progesterone receptor positivity (HR=0.77, 95% CI=0.51-1.03). Independent predictors of overall survival at the time of development of metastatic disease included progesterone receptor positivity (HR=0.62, 95% CI=0.26-0.98), prior adjuvant chemotherapy (HR=2.34, 95% CI=1.94-2.74), prior adjuvant hormone therapy (HR=2.33, 95% CI=1.83-2.83), disease-free interval (HR=0.98, 95% CI=0.97-0.99), the presence of bone metastases (HR=0.59, 95% CI=0.17-1.01), the presence of locoregional disease (HR=0.45, 95% CI=0.02-0.88), the presence of liver metastases

(HR=1.68, 95% CI=1.08-2.12), and the number of sites of disease (HR=2.06, 95% CI=1.78-2.34).

Conclusions: The use of HDCT/AHPCT in metastatic breast cancer may confer advantages with respect to overall survival and time to first failure after beginning chemotherapy. These advantages appear to be independent of the effects of selection bias and variously cited prognostic factors. This benefit if confirmed in ongoing randomized trials will have to be considered in light of differences in cost and quality of life between the two therapeutic modalities.

Acknowledgements

I would like to thank Dr. S. Raman and Dr. S Gluck for assistance with the development and preparation of this thesis, particularly for their unending patience throughout this learning process. I would also like to thank Drs. Gluck, Reed, and Tarantolo for assistance with obtaining the datasets for the experimental groups. Thanks to Jene Pierson (University of Nebraska Medical Center) and Terry Koski (Northeastern Ontario Regional Cancer Centre) for assistance with formatting and obtaining the datasets.

Thanks to Paul Hebert for assistance with the development of this thesis.

Thanks to Lisa Lipknowski for helping to review and screen charts, and to Judy Morris and the entire Ottawa Regional Cancer Centre medical records staff for assistance with obtaining charts for review.

To Lothar Huebsch for being part colleague, part mentor, and constant friend. Thanks for always helping me do the right thing.

To Amy, for patience, for learning to love hockey and life in Canada, and for tolerating me as I tried to keep the lawn mowed while completing this degree.

To my parents, for giving me the skills which have allowed me to become the best person I can be, and in particular for their patience and understanding when I wavered onto paths of less certainty.

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I. Introduction/Background

Carcinoma of the breast is the most common malignancy in North American women, accounting for 27% of all cancers, and 18% of cancer related deaths in females.[1] For women between the age of 40 and 55 years, breast cancer is the leading cause of death in North America.[1] The estimated age-standardized incidence of breast cancer is presently 110/100,000 females and appears to be increasing annually at a rate of approximately 1.7 percent per annum.[2] Present evidence and trends would suggest that the average North American woman has a lifetime risk of developing breast cancer of one in nine.[2]

The most clearly demonstrated risk factors for the development of breast cancer include a prior family history, especially among first degree relatives,[3,4] a younger age at menarche,[5,6] older age at menopause,[7] older age at first full term pregnancy,[8,9] and the past use of exogenous hormones (estrogen replacement therapy, oral contraceptives).[10-12] Much recent work has focused on the identification of mutations in certain genes which, when present appear to increase the risk of breast and ovarian cancer substantially,[13,14] and carry a heritable risk of transmission to the offspring of affected individuals. Risk factors which have been more controversial in the literature include a diet high in fat,[15,16] and the consumption of alcohol.[17,18] Unfortunately, the potentially modifiable risk factors (e.g., diet, alcohol consumption) likely contribute only a very modest increment in relative risk, with quantitatively more important risk factors such as family history/genetics being unmodifiable. It seems likely therefore that for the average individual, alterations in dietary measures or other lifestyle changes will not have a significant impact on the eventual development of breast cancer.

i) Treatment of Primary Breast Cancer

Patients who have no overt evidence of distant metastases at initial diagnosis (i.e., no evidence of cancer beyond the breast and ipsilateral axillary lymph nodes) have a prognosis and risk of future recurrence which depends principally on factors such as primary tumor size,[19-22] the presence or absence of axillary lymph node involvement with tumor,[23-27] the histologic or nuclear grade of the primary tumor,[20,28,29] and the presence or absence of estrogen and progesterone receptors in tumor tissue.[30-32] Tumor size, nodal status, and the presence or absence of distant metastases combine to form a clinical stage which is used for descriptive, prognostic and therapeutic purposes (appendix 1). For patients with a resectable tumor and no evidence of metastatic disease, initial management consists of surgery to remove all visible disease and to provide pathologic staging information (i.e., to establish the size of the primary tumor and the presence or absence of axillary lymph node involvement). This most often consists of either 1) a modified radical mastectomy (removal of the entire breast, pectoralis minor muscle, and axillary lymph nodes), or 2) a breast conserving surgical approach (lumpectomy, or the removal of the malignant tumor plus a variable margin of normal surrounding tissue, also accompanied by an axillary lymph node dissection). Since breast conserving approaches do not remove the entire breast, the probability of residual microscopic foci of malignancy within remaining breast tissue is high, and lumpectomy is therefore generally followed by local radiotherapy to the remaining breast tissue to reduce the risk of local recurrence. Since this additional local therapy is given in the absence of any known or proven residual disease, the term "adjuvant" is commonly used. A number of randomized trials[33,34] as well as recent meta-analyses of randomized controlled trials[35-37] have demonstrated that both of these methods are associated with an equal probability of long term survival. The choice of local surgical

approach therefore depends largely on patient preference and occasionally on surgically related technical factors.

In occasional cases adjuvant radiotherapy may also be offered to patients who have undergone a modified radical mastectomy, as a limited and somewhat controversial literature supports its superiority over surgery alone.[38,39]

Where the risk of future systemic recurrence is considered to be clinically important to a patient and physician, patients are generally offered some form of systemic adjuvant therapy in an attempt to eliminate potential occult distant microscopic foci of disease and increase the probability of cure. Systemic adjuvant therapy generally consists of some form of cytotoxic chemotherapy, hormonal therapy, or both. The choice is influenced by primary prognostic factors such as those previously indicated. The use of systemic adjuvant therapy in numerous randomized trials,[40,41] and recent meta-analyses[42,43] consistently results in a demonstrable relative risk reduction (for systemic recurrence) of approximately one third. Despite administration of the best available primary local and systemic therapy, approximately one third of patients with breast cancer will suffer a recurrence of their disease during their lifetime.[44] Breast cancer is an extremely heterogeneous disease, and as such the interval from diagnosis to first recurrence is highly variable. It is not uncommon for patients to suffer a relapse of their illness 10 or more years after initial diagnosis and therapy.[45-47]

ii) Metastatic Breast Cancer

Patients who present with, or who eventually develop distant metastatic disease are incurable with present conventional chemotherapy (CCT), and have a median survival of approximately two to three years.[48-51] The most common sites of distant recurrence are bone, liver, lung, lymph nodes, and brain.[52,53] Metastatic breast cancer, like the primary illness, displays a wide range of biologic variability. Although some patients survive only weeks after the development of clinically overt metastatic disease, ten to forty percent of patients may live for up to five years or more,[48,54-56] and occasional patients may live ten years or more after the diagnosis of metastatic disease.[48,50] The use of conventional systemic therapy (chemotherapy and/or hormone therapy) can lead to a clinically measurable reduction in tumor mass, which for many patients leads to improvement, often substantial, in symptom control and overall quality of life,[51] although any prolongation of survival resulting from therapy is less clear.[57,58] The inability to clearly document the impact of treatment on survival in metastatic breast cancer results from a lack of (for obvious ethical reasons) randomized controlled trials in this area. Most studies attempting to measure a survival impact resulting from treatment have used indirect methods of outcome measure. For example some studies have compared changes in survival using older temporal cohorts (e.g., prior to the availability of chemotherapy) as control groups,[55] but this type of study design lacks the ability to adjust for potential temporal differences in the disease over time, and likely also suffers from a form of lead-time bias resulting from enhanced diagnostic/disease detection methods in the more temporally current patient groups. This lead-time bias would tend to favour the observation of a survival advantage in the treated (more recent) patient populations. Using comparisons of "responders" and "non-responders" is another widely used method of indirectly comparing the effects of treatment on survival,[59] but this method overlooks a bias related to the biology of the disease (i.e., that response to

treatment is in itself a confounding variable, or prognostic factor which predicts for a longer survival independent of the form of therapy).[60] Since survival advantages resulting from treatment have been difficult to establish, the principal goals in treating patients with metastatic disease have been to improve symptoms caused by the presence of the tumor, and to prolong the period of disease control, as hopefully achieving these endpoints will improve the overall quality of life of the patient.

iii) Prognostic Factors in Metastatic Disease

Over the past two decades, retrospective and prospective studies of women with metastatic breast cancer have allowed clinicians to elucidate several prognostic factors which have been used as predictors of both response to therapy and survival. In general terms these prognostic factors relate to clinical features at diagnosis, measures of disease bulk and biology at recurrence, the use of past systemic therapy, and the response to systemic therapy given after recurrence. Lionetto et al[50] analyzed patterns of survival in 302 patients with metastatic disease. Univariate analysis revealed that a shorter disease-free interval, the presence of visceral metastases, and prior adjuvant chemotherapy were all associated with a shorter survival with metastatic disease. Clark et al[46] performed a retrospective analysis of 1,015 patients treated at the University of Texas Health Science center between 1971 and 1983 in an attempt to identify prognostic factors influencing survival after the development of metastatic disease. Variables studied included initial clinical stage, estrogen receptor status, prior treatment with adjuvant chemotherapy or hormone therapy, disease-free interval, age at relapse, and number and location of recurrent sites of disease. Multivariate analysis using the Cox proportional hazards model[61] identified a longer disease-free interval and estrogen receptor positivity to be associated with improved survival, while the presence of brain, lung, liver, and bone metastases were each independently associated with poorer

survival. The Eastern Cooperative Oncology Group performed a similar retrospective analysis on data from 1,168 patients with metastatic breast cancer.[62] Among 18 potential prognostic factors studied, younger age, better performance status, fewer sites of disease, and absence of visceral metastases were all identified as all being independent predictors of longer survival. Other retrospective studies of large databases have confirmed in multivariate analyses the importance of variables such as bulk of disease, sites of recurrence, disease-free interval, estrogen receptor status, and where investigated, tumor grade.[59,63] Prospectively conducted clinical trials which have sought to measure independent prognostic factors for survival with metastatic disease confirm the importance of variables such as disease-free interval, number of sites and location of recurrence, hormone receptor status of the primary tumor, and a history of prior adjuvant therapy.[64,65]

Given that therapy does not appear to substantially alter survival for this group of patients, the observed differences in survival for patients with metastatic disease are likely largely a reflection of inter-patient differences in the previously discussed prognostic factors at the time of recurrence. This intrinsic wide biologic variability has hampered the evaluation of new therapies, especially when study designs have either not incorporated randomized control groups, have not recognized/adjusted for potential prognostic differences between groups after randomization, or have not designed the study with the appropriate statistical power to detect a modest difference in the clinical outcome under study.

iv) Treatment of Metastatic Breast Cancer

Since therapy for metastatic breast cancer does not appear to confer any significant survival advantage for the average patient, therapy is generally symptom based, with attempts to minimize systemic toxicity until the point at which more simple therapies have failed. For example radiotherapy can be used to achieve local symptom control (e.g., radiation to sites of painful bone metastases or nodal metastases) with few or no side effects being experienced by the patient. Because of their low toxicity profile, hormonal therapies (e.g., estrogen receptor antagonists, aromatase inhibitors) are often the first form of systemic therapy attempted, particularly for patients whose tumors are hormone receptor positive, and who have predominantly bone and soft tissue (e.g., lymph node) metastases. Although many patients (particularly those with receptor positive tumors) may initially respond to hormonal manipulation, hormonal resistance and disease progression invariably develop after a period of time, generally within 12 to 20 months.[51,66-71]

Metastatic breast cancer no longer responsive to hormonal therapy is most often treated with CCT using either single agents or combinations of agents.[1,58,64,72-74] Initial responses to chemotherapy are generally seen in 45 to 80% of patients, although complete responses (i.e., complete remissions) are uncommon, generally being seen in 5-20% percent of patients.[54,56,64,73,74] The median duration of response is between 5 and 13 months, and the median survival between 1 and 3 years.[1] Despite the introduction of new chemotherapeutic agents in recent years, any apparent progress in the treatment of metastatic breast cancer has been marginal at best, an observation which has lead to the pursuit of more aggressive forms of therapy.

v) Therapeutic Failure and the Rationale for Dose-Intensification

The inability to cure a particular cancer with conventional doses of chemotherapeutic agents is generally ascribed to the acquisition of drug resistance by certain cells within the tumor. The mechanisms by which resistance can develop are several, and have been well described.[75] Many strategies aimed at overcoming these specific forms of resistance have been reported on[75] and will not be reviewed in detail here. However, one conceptually simple means by which certain resistance mechanisms might be overcome is through dose-intensification.

The concept of dose-intensification originates in the demonstration of what has generally been referred to as a dose-response relationship. Certain tumors, including breast cancer [76-78] appear to demonstrate a greater magnitude of response to increasing doses of chemotherapy. By greatly increasing the dose(s) of drug(s) delivered to the patient, dose-intense therapy provides a means by which resistance mechanisms in malignant cells might be overcome. The mechanisms by which dose-intensification overcomes some forms of drug resistance have not been well elucidated, but appear to relate to factors such as overwhelming the malignant cell's ability to either inactivate the drug, or to repair damage to DNA prior to the next replication cycle.[79,80] If a dose-response relationship can be demonstrated for a particular tumor, the degree to which one can expect to achieve meaningful clinical benefit from dose-intensification depends on the slope of the dose-response relationship (Fig 1).

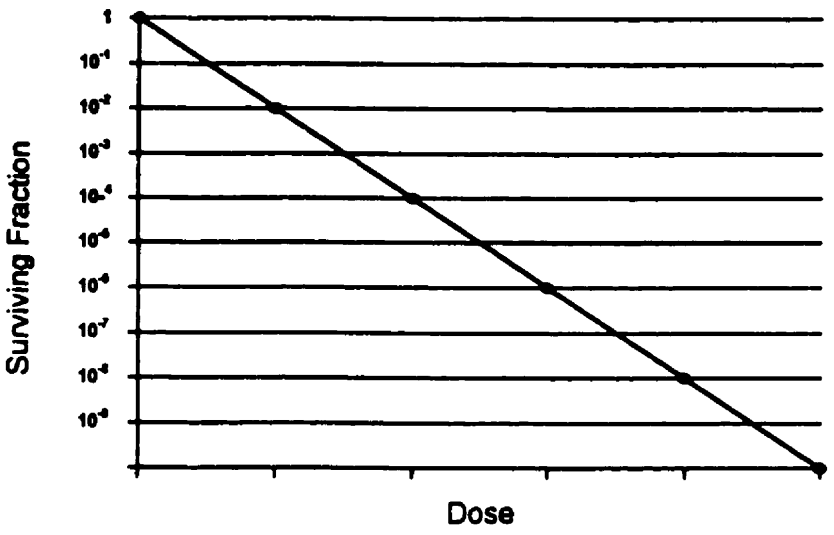


Fig 1. The theoretical relationship between the dose of a chemotherapeutic agent administered and the surviving fraction of cells. The Y-axis represents the proportion of cells surviving after exposure to a hypothetical chemotherapeutic agent, and the X-axis represents incremental doses of that agent. As dose increases, the proportion of surviving cells decreases. The slope of the curve indicates the relative degree of dose-responsiveness for a particular tumor, with a decreasing slope representing a greater degree of dose-responsiveness.

vi) Evidence for a Dose-Response Relationship in Breast Cancer

Evidence for the existence of a dose-response relationship in breast cancer comes from both retrospective data analyses, and from prospectively conducted randomized clinical trials, and includes data from patients treated in both the adjuvant and metastatic settings. The earliest attempt to analyze the effect of dose on outcome in breast cancer came from a retrospective analysis conducted by Bonadonna and Valagussa.[78] This study retrospectively analyzed relapse-free survival at five years in 901 women with breast cancer who had been treated with chemotherapy as part of three earlier prospective clinical trials.[81-83] Since dose reductions are common during CCT, they reviewed the actual total doses which had been received by each patient in their trials, and expressed these as a percentage of the intended full (planned) doses. Among 348 pre-menopausal patients receiving adjuvant chemotherapy in this study, those who had received ≥ 85% of the intended dose had the best five year relapse-free survival (79.1%), followed by patients who had received 65-84% of the planned dose (55.7%), followed by patients who had received less than 65% of the planned dose (43.4%). The results were similar, though less marked for the 280 post-menopausal patients. Not all between-group differences were statistically significant (particularly in the post-menopausal group). The statistical analysis of this data was somewhat weak. More information may have been forthcoming had the investigators perfomed a single life-table analysis of all treated patients (rather than breaking the patients into menopausal subsets), or by performing a multivariate analysis, including variables such as menopausal status and proportionate planned dose received as covariates in the analysis. However, the appearance of the groups as projected by life table analysis suggests graphically that a dose-response effect is a more likely explanation than a simple threshold effect (i.e., where patients below a certain threshold dose obtained no clinical benefit whatsoever).

Hryniuk and Bush [77] performed a retrospective analysis of dose-intensity and outcome in patients receiving chemotherapy for metastatic breast cancer. Using the dose-intensity (expressed as doses of drugs received in mg/m^2/week) of the Cooper regimen[84] as a reference, the average relative dose-intensities of several other published trials using cyclophosphamide, methotrexate, and fluorouracil (CMF) were calculated and were assigned a fractional dose-intensity relative to the Cooper regimen. The relationship between the average relative dose-intensity and outcome (the published response rate for each particular study) was then explored. The authors concluded that a relationship between dose-intensity and response could be demonstrated ($r=0.82$, $P<0.001$). The same analysis was performed for published reports using cyclophosphamide, doxorubicin, and fluorouracil (CAF) as the chemotherapy regimen, using the regimen of Bull and Tormey[85] as the reference dose-intensity regimen. This analysis revealed a similar relationship between average relative dose-intensity and response rate ($r=0.71$, $P<0.01$). Although the authors acknowledged the limitations of their design, particularly the use of average relative dose-intensities and published response rates to generate their conclusions, the study is considered by most investigators to have contributed some data favoring of the existence of a dose-response relationship in breast cancer.

Two important prospective studies have contributed information to the issue of dose-responsiveness in breast cancer. A prospective trial conducted by Tannock et al[76] randomized 133 patients with metastatic breast cancer to one of two dose levels of CMF (cyclophosphamide 300 mg/m^2, methotrexate 20 mg/m^2, and fluorouracil 300 mg/m^2 or cyclophosphamide 600 mg/m^2, methotrexate 40 mg/m^2, and fluorouracil 600 mg/m^2, each delivered on a repeating 21 day cycle). Patients randomly assigned to receive the higher dose levels of CMF had a higher response rate compared to patients receiving the lower dose levels (response rate 30% vs. 11%, $P=0.03$). In univariate

analysis the group randomized to the higher dose levels also had a longer median survival compared to the group receiving the lower dose levels (median survival 15.6 months vs. 12.8 months, P=0.026 by logrank), although because of random between-group differences in certain prognostic factors the statistical significance of this survival difference was not maintained in multivariate analysis (p=0.12 using Cox proportional hazards model). Patients in this study not responding to the lower dose levels were crossed over to the higher dose levels, but of 37 patients crossed over to the higher dose levels, only one patient responded, and as such, this likely did not affect the outcome of the trial significantly. The authors did not discuss how this group of crossover patients was dealt with in the analysis (e.g., by censoring outcomes at the point of crossover). The small sample size of this study likely precluded the ability to find significance in such a small observed (< 3 months) difference in median survival.

Finally, a prospective clinical trial conducted by the Cancer and Leukemia Group B, reported by Wood et al[86] randomized 1572 women with operable breast cancer to receive one of three dose-intensities of adjuvant CAF. After a median of 3.4 years follow-up, patients who had received either of the two more dose-intense levels of CAF had significantly longer disease-free survival (p<0.001) and overall survival (P=0.004) compared to those randomized to the lowest level of CAF. However, the difference in outcome between patients receiving the two higher levels of CAF was not statistically significant. Again, although a clear linear relationship between dose and outcome could not be unequivocally established, this trial lends some support to the concept of dose- intensity.

In summary, the demonstration of a clear dose-response relationship in breast cancer in both the adjuvant and metastatic settings has been difficult, and in both retrospective and prospective studies, the data supporting the existence of such a relationship has

been weak in general. However, it is important to consider that some of these studies may have been hampered by relatively simple problems such as sample size/power issues, and that all of these studies have dealt only with conventional levels of chemotherapeutic dose escalation (i.e., dose differences of generally less than two fold). Therefore the possibility of achieving further clinical benefit from raising dose levels several fold cannot be excluded on the basis of this type of data. This clinical hypothesis is supported by the observation that resistance to alkylating agents (which are commonly used in high-dose chemotherapy regimens) can be overcome in both laboratory and animal models by raising the dose levels by 5 to 10 fold.[79,87]

Since the effects of chemotherapy are relatively non-selective (i.e., cause some degree of damage to both tumor cells and to normal host tissues/organs), the concept of significant dose escalation poses potential clinical problems. The tissues/organs most sensitive to the effects of chemotherapy are those which are rapidly dividing, such as the gastrointestinal tract, bone marrow, and skin. In considering the escalation of conventional drug doses by 5-10 fold, it would be expected that these organs would be subjected to a substantially increased degree and duration of toxicity. In particular, the bone marrow (which is the source of hematopoiesis or the generation of blood and immune cells) is the dose-limiting organ for most chemotherapeutic agents. As one escalates the dose(s) of drug(s) delivered, the duration and degree of subsequent bone marrow suppression increase substantially, resulting in an increased period of risk to the patient (in particular from life-threatening infection and bleeding, resulting from lowered white blood cell counts and platelet counts respectively). These risks could potentially be lowered through the use of some physical and/or pharmacologic form of hematopoietic support which could reduce the duration of bone marrow suppression resulting from high-dose chemotherapy.

vii) The Use of Hematopoietic Progenitor Cell Support for Patients Undergoing Dose-Intensified Therapy

The earliest report of therapeutic marrow infusion came in 1939 involving a patient who received 18 ml of intravenous marrow from his brother as a treatment for aplastic anemia.[88] Following the use of the atomic bomb at the end of World War II, interest in radioprotection prompted experiments demonstrating that mice could withstand otherwise lethal exposure to total body irradiation by shielding the spleen (part of the hematopoietic system) [89] or by infusing marrow post exposure.[90] In 1959 hematologic recovery following syngeneic (identical twin) marrow transplantation for leukemia demonstrated that a compatible marrow graft could rescue a human from the effects of lethal irradiation.[91] These experiments suggested that hematopoietic progenitor cells with the potential for long term function could be harvested from the bone marrow compartment and re-infused into individuals after they had received intense therapy to treat their disease. Recognition of the importance of genetic compatibility, of certain transplantation antigens (the Human Leukocyte Antigen loci) and the development of potent immunosuppressive agents allowed investigators to subsequently develop means by which marrow from genetically non-identical individuals (allogeneic) could be used as the source of hematopoietic reconstitution.

viii) Sources of Hematopoietic Progenitor Cells

The initial source of hematopoietic progenitor cells for transplantation was the bone marrow, which could safely be harvested from an individual under general anesthetic from the pelvic bones.[92] It was later discovered that hematopoietic progenitor cells capable of long term engraftment circulate at low levels in the peripheral blood, and that these cells could be collected (removed from the veins through a procedure known

as apheresis) without the need to harvest bone marrow. Initially these cells were collected without any attempt to increase their circulating number (non-mobilized), however numerous aphereses were required.[93] Subsequently, chemotherapy,[94] hematopoietic growth factors,[95] or both,[96] have been used to augment the number of circulating progenitor cells for transplantation such that an adequate number of cells for a transplant can now often be collected with a single apheresis.[97] Potential advantages of peripheral blood progenitor cells include collection without the need for general anesthesia or repeated painful bone marrow aspirations, diminished contamination with tumor cells,[98,99] accelerated hematologic recovery,[100] particularly for platelets,[101] and perhaps some degree of immunomodulatory anti-tumor activity compared to bone marrow.[102] As confidence with the long-term engraftment capability of peripheral blood has grown, its use has generally expanded in most North American transplant centres.

ix) Autologous Transplantation versus Allogeneic Transplantation

Based on the source of progenitor cells used, transplantation is considered to be autologous or allogeneic. In autologous transplantation, the re-infused progenitor cells can come either from the patient's bone marrow (ABMT) or blood (peripheral blood progenitor cell transplantation, PBPCT). The major advantages of autologous transplantation include the ready availability of a donor (the patient) for the progenitor cell product, the absence of the need for immunosuppressive drugs (to allow the progenitor cell product to engraft in a genetically non-identical environment), and the absence of an illness known as graft-versus-host disease (discussed below). In allogeneic transplantation, the re-infused progenitor cells are derived from the bone marrow or peripheral blood of a genetically identical or genetically similar donor. The major advantages of allogeneic transplantation include the absence of tumour cells within the graft (a recognised potential source of post-transplant relapse in the

autologous setting),[103,104] and the potential for what is known as the graft-versus-tumour effect.[105] It has been consistently observed that patients with the same illness who undergo allogeneic transplantation (compared to autologous transplantation) have a lower incidence of relapse of the primary disease.[106,107] The hypothesis is that immunocompetent donor immune cells within the allograft can recognise host tumour cells as being foreign, destroying them immunologically. Unfortunately the same immunocompetent donor cells also often recognise other normal host tissues as foreign, and can cause immunologically mediated injury to these tissues (in particular, tissues from the gastrointestinal tract, liver, and skin); an illness known as graft-versus-host disease. When severe, graft-versus-host disease can be fatal.[108] In the future, identification/recognition of those subsets of T-cells responsible for graft-versus-host disease could allow allogeneic grafts to be "engineered" in such a way as to minimise graft-versus-host disease, while maintaining the graft-versus-tumour effect.

x) Early Studies of High-Dose Therapy with Autologous Hematopoietic Progenitor Cell Transplantation in Metastatic Breast Cancer

Using the traditional approach to developing new clinical therapies, early phase I-II studies of high-dose chemotherapy/autologous hematopoietic progenitor cell transplantation (HDCT/AHPCT) for metastatic breast cancer were offered only to patients in whom no other conventional therapy was deemed likely to be of benefit. As such, patients generally had poor functional status, poor major organ function, and tumors that were refractory to CCT. Despite this selection of a very poor prognostic subset of patients, results from the largest of these early trials suggested that a meaningful proportion of such patients could respond to high-dose chemotherapy (table 1).[109,110] However it was consistently observed that complete responses were rare, and response durations were short, generally in the range of three to four months. Though

interpretation is limited by the heterogeneity of these studies, trials using combinations of chemotherapy agents appeared to have higher response rates than trials using only single agents.[111] Since patient selection for these early trials was directed at those who had been heavily pretreated, mortality rates of up to 20% were observed.[109]

Institution	Agents	N	CR (%)	RR (%)	Median Response Duration	Toxic Deaths (%)	Reference
MDA	MMC	15	0	40	< 3 months	20	109
MDA	CVP	32	23	61	4 months	6	110
MDA	AMSA	16	0	13	< 4 months	0	112
UC	CT	14	15	77	3 months	6	113
D-F	CBP	56	0	81	3 months	18	114

MDA=MD Anderson Hospital; UC= University of Chicago; D-F=Dana-Farber; MMC=mitomycin-C; AMSA= amsacrine; CT=cyclophosphamide, thiotepa; CBP=cyclophosphamide, carmustine, cisplatin; CVP=cyclophosphamide, etoposide, cisplatin

The consistently reproducible observation of greater than expected response rates from these early studies led investigators to bring this therapy to a better prognosis group of patients; those who had not received prior chemotherapy for metastatic disease, since they would be expected to be more tolerant of therapy, and achieve better response rates on the whole than patients known to be refractory to CCT. Patients generally received a number of cycles of initial (induction) CCT, followed by HDCT/AHPCT. The largest trials enrolling patients of this type reported overall response rates of approximately 70-90%, with complete responses being seen in up to

50% of patients (table 2).[115-118] The median durations of response were generally in the range of 7-10 months,[116,117] with toxic death rates between 5 and 30%.[118] Two additional important clinical observations came from this series of trials. First, it was noted that many patients who had achieved only a partial response to induction chemotherapy went on to complete responses after HDCT/AHPCT,[118,119] suggesting that chemo-resistance could indeed be overcome at higher doses. Second, it was observed that a proportion of patients treated with HDCT/AHPCT appeared to be achieving stable long term disease control, with up to 15-20% of patients being free from evidence of progression or recurrence at periods of 2-4 years post treatment,[116,117,120] hinting at the possibility that some of these patients might be cured by HDCT/AHPCT.

[redacted]

Institution	Agents	N	CR (%)	RR (%)	Median Response Duration	Survival	Toxic Deaths (%)	Reference
D-F	CBP	15	38	88	5 months	30% 3 years (projected)	18	115
UC	CT	22	58	70	10 months	50% 2 years	5	116
Duke	CBP	22	54	73	9 months	40% 4 years	13	117
UC	CTB	45	44	71	7.5 months	25% 3 years	30	118

D-F=Dana-Farber; UC= University of Chicago;
CBP=cyclophosphamide, carmustine, cisplatin; CT=cyclophosphamide, thiotepa; CTB=cyclophosphamide, thiotepa, carmustine

Pushing phase II studies to their limit, the next series of studies included the selection bias of treating only patients who had responded to induction therapy, based on the constant oncologic observation that "responders" have the best outcomes with any particular therapy.[60] As expected, the largest of these trials showed high overall response rates of 85-100%, with complete responses being seen in 45-59% of patients (table 3).[121-123] Median time to progression was between 7 and 13 months. Again, a number of partial responders became complete responders after HDCT/AHPCT, and a number of long term responders were seen, with 6-17% of patients free of progression up to 5 years after treatment.[121,122]

Table 3: High-dose Chemotherapy/Autologous Hematopoietic Progenitor Cell Transplantation in Responding Metastatic Breast Cancer

Institution	Agents	N	CR (%)	RR (%)	Median Response Duration	Survival	Toxic Deaths (%)	Reference
D-F	CTCb	29	45	100	10 months	30% 3 years	3	121
IHHSL	CMM	61	59	85	13 months	36% 5 years	12	122
RM	M	15	46	93	7 months	20% 2 years	20	123

D-F=Dana-Farber; IHHSL=Institute d'Hematologie, Hospital St. Louis; RM=Royal Marsden; CTCb=cyclophosphamide, thiotepa, carboplatin; M=melphalan; CMM=cyclophosphamide, melphalan, mitoxantrone

Finally during this period, various investigators reported on several related issues. These included attempts to prolong responses by administering multiple cycles of HDCT/AHPCT,[124,125] moving more toward peripheral blood as the source of hematopoietic progenitor cells,[126] moving treatments into the outpatient setting,[127]

measuring health related quality of life issues,[128] and attempting to identify prognostic factors associated with outcome in this setting.[129-132]

In summary, a long and reasonably thoughtful evolution of phase I-II trials of HDCT/AHPCT in metastatic breast cancer revealed much about the potential benefits of the treatment, but a lack of strong clinical science in trial designs, and the lack of any randomized controlled trials had left further progress in this area stalled. Ongoing randomized trials were beginning to have difficulty accruing patients because of strong patient and physician biases favoring the use of HDCT/AHPCT. Interestingly, at this time an alternate group of breast cancer physicians were beginning to express a strong negative view of this form of treatment. They claimed that the seemingly positive results of phase I-II trials in this area could alternatively be attributable to selection bias. This concept was very elegantly presented and quantified by researchers from the University of Texas MD Anderson Cancer Center at a major international oncologic meeting in Los Angeles in May 1995.[133] The authors presented a carefully conducted retrospective review of the potential effect of selection bias on the outcome of patients with metastatic breast cancer treated with chemotherapy. This presentation utilized prospectively collected data on 1581 patients with metastatic breast cancer enrolled in previous doxorubicin-containing clinical trials. Using common eligibility criteria for HDCT/AHPCT the authors determined retrospectively which patients they felt would have been candidates (or non-candidates) for this type of therapy. Response rates, progression-free survival, and overall survival were analyzed and compared between those considered "candidates" and those who were "non-candidates". The results clearly demonstrated that those patients who would have been selected as candidates for HDCT/AHPCT had a better outcome than those who would not have been candidates, and that this outcome appeared to be attributable entirely to selection bias. The inference was that the usual selection criteria for transplant studies identified a

group of patients with a better prognosis independent of therapy. In this study, the factors that accounted for the exclusion of candidates in the majority of cases were age, performance status, and response to chemotherapy. This study again brought to light the subtle biases and limitations of uncontrolled studies, and was subsequently accepted for publication in a major peer reviewed journal.[134]

xi) A Randomized Trial of High-Dose Therapy with Autologous Progenitor Cell Transplantation in Metastatic Breast Cancer

The first randomized controlled clinical trial comparing HDCT/AHPCT to CCT was published in a peer-reviewed journal by Bezwoda et al from the University of Witwatersrand in South Africa in October 1995.[135] The objectives of the study were to compare response rates, duration of response, and duration of overall survival between groups. Eligibility required patients to be ≤ 50 years of age with histologically or cytologically confirmed metastatic breast cancer, no prior chemotherapy for metastatic disease, adequate end organ function as determined by standard biochemical and imaging studies, and an Eastern Cooperative Oncology Group performance status of 2 or better (appendix 2). Randomization was performed by a random number closed envelope technique. Between January 1991 and February 1993, 90 patients were randomized to receive either 6 cycles of CCT (cyclophosphamide 600 mg/m^2, mitoxantrone 12 mg/m^2, vincristine 1.4 mg/m^2 every three weeks) or to two cycles of HDCT/AHPCT (cyclophosphamide 2.4 g/m^2, mitoxantrone 35-45 mg/m^2, VP-16 2.5 g/m^2, repeated once at day 42). Patients were re-evaluated after each two treatment cycles of CCT, or at four weeks after each cycle of high-dose chemotherapy. Patients randomized to CCT who had objective evidence of tumor regression at 6 cycles received two additional cycles of the same chemotherapy. All responding patients in the trial received tamoxifen 20 mg orally daily until objective signs of progression.

In this trial, the response rate for patients randomized to HDCT/AHPCT was 95%, with a complete response rate of 51%, compared to a 53% response rate and 5% complete response rate for the control group (P<0.01). The median duration of response for patients randomized to HDCT/AHPCT was 80 weeks compared with 34 weeks for the control group (P not given). Finally, the median duration of survival for patients randomized to HDCT/AHPCT was 90 weeks compared with 45 weeks for the control group (P not given). The authors concluded that their high dose chemotherapy regimen appeared to be "a promising schedule that results in a significant proportion of complete responses and increased survival in patients with metastatic breast cancer". For several reasons that will be discussed in a later section, this trial was considered by most investigators to have lent some further credibility to the benefits of HDCT/AHPCT for this population, but not to have answered the question conclusively because of several methodologic limitations.

At the present time, several other randomized trials to evaluate the potential benefit of this form of therapy are being conducted,[136] but because of ever increasing physician/patient biases toward high-dose therapy some of these trials may in fact not be able to be completed. In the best scenario, further mature randomized data is likely to be 3-5 years away. Nonetheless, there is a potential wealth of information contained in transplanted patients to date, and an appropriately designed retrospective analysis could still yield some important comparative prognostic and outcome information regarding the effect of HDCT/AHPCT.

II. The Hypothesis

The hypothesis is that a clinically demonstrable dose-response relationship may exist for metastatic breast cancer, and that the existence of this relationship could be demonstrated by comparing relevant clinical outcomes for two forms of therapy (CCT as compared to HDCT/AHPCT) in patients with metastatic breast cancer. The most easily measured clinical outcomes would be the duration of overall survival and the duration of freedom from relapse/progression (time to failure). Implicit in this hypothesis is the assumption that both groups of patients are comparable with respect to the potential effect of various forms of selection bias, and that significant confounding variables which could affect outcome have been measured such that their effect can be adjusted for in the analysis.

III. Objectives

The principal objectives of this study are to compare the duration of overall survival and time to first failure after beginning chemotherapy in patients with recurrent/metastatic breast cancer as influenced by the type of therapy received: HDCT/AHPCT versus CCT. Secondary endpoints include 1) a confirmatory analysis of prognostic factors for time to recurrence, and prognostic factors in patients with metastatic disease, and 2) in the HDCT/AHPCT group, measurement of the effects of factors such as treatment centre, the actual high-dose chemotherapy regimen used, the presence of bone marrow involvement with tumor at transplantation, and the source of progenitor cells (bone marrow versus peripheral blood) if possible on the above endpoints.

IV. Study Design/Methodology

i) Principal Study Design

This study was designed as a comparative (retrospective cohort) analysis of two groups of patients with metastatic breast cancer; one group having received HDCT/AHPCT for their disease (the exposure, or experimental therapy group), and one group having received modern CCT with no progenitor cell support (no exposure, or control group). The period of time chosen for the study was January 1991 to December 1995 such that a minimum of two years of follow-up time could have been observed for all patients in both groups. Patients were identified as study candidates using predetermined selection/eligibility criteria (discussed later).

ii) Source of Subjects

1) Experimental Patients

The experimental group was compiled from two existing separate datasets. The first experimental dataset consisted of patients who received HDCT/AHPCT as part of a prospective phase II clinical trial run at the University of Nebraska Medical Centre (UNMC). The principal investigators for this study were Drs. Elizabeth Reed and Stefano Tarantolo. The second experimental dataset consisted of patients who received HDCT/AHPCT in four sequential prospective phase I or phase I-II trials at the Northeastern Ontario Regional Cancer Centre (NEORCC). The principal investigator for these trials was Dr. Stefan Gluck.

2) Control Patients

The Ottawa Regional Cancer Centre (ORCC) has a wide referral base of approximately one and one half million people, and sees approximately 800 new cases of breast cancer annually (internal data). After obtaining appropriate internal institutional consent to collect ORCC patient related information, the population from which the control group would be selected was identified. This population was defined as all patients referred to the ORCC who had been diagnosed with metastatic breast cancer and who had received chemotherapy for metastatic disease at some point during the chosen time period for the study. This population was generated using a computerized search strategy within the Oncology Patient Information System (OPIS). This population was then sampled through individual patient chart review to determine those patients who would have been eligible for HDCT/AHPCT, but who received CCT for their illness (the control group). The inclusion criteria were designed to approximate as closely as possible the same physiologic/biologic type of patient as was represented by the experimental group to minimize the effects of selection bias. Also, to minimize the introduction of any possible bias incurred by a temporal or sequenced selection of patients, chart numbers were selected at random using numbers generated from a random number table (generated using the random number function in Microsoft Excel).

iii) Summary of Eligibility Criteria for Experimental and Control Patients

The following is a summary of eligibility requirements common to all patients. Specific eligibility requirements for each treatment group may be found in appendices 4, 5, and 6.

1) Disease Characteristics/Demographics

Patients were required to have initial histologic confirmation of a diagnosis of breast cancer with evidence of recurrent or metastatic disease, either by biopsy, or by appropriate imaging studies (e.g., bone scans, CT scans showing unequivocal evidence of metastatic disease in the treating physician's opinion). There were no restrictions on gender. Patients had to be between 18 and 60 years of age. Patients could be pre or post menopausal. Patients were allowed to have received prior systemic adjuvant therapy (chemotherapy, hormonal therapy, or both). Initial estrogen/progesterone receptor status was recorded where known. An Eastern Cooperative Oncology Group performance status (appendix 2) of 0-2 was required for eligibility.

2) Physiologic Status/ Major Organ Function

Patients were required to have adequate major organ function as dictated by the following parameters:

Renal function: Serum creatinine < 120 mmol/L (1.5 mg/dl)

Hepatic function: Serum bilirubin < 26 mmol/L (2 mg/dl), and AST/ALT < 2x normal unless due to metastatic disease

Pulmonary function: Flow rates/diffusing capacity ≥75% normal

Cardiac function: Left ventricular ejection fraction ≥ 50%

If any prior cardiotoxic anthracyclines, the total dose received must have been ≤ 450 mg/m^2 for doxorubicin, and ≤ 800mg/m^2 for epirubicin.

Hematologic function: White blood cell count ≥ 3.5 x 10^9/L

Absolute neutrophil count ≥ 1.5 x 10^9/L;

Platelet count ≥ 100 x 10^9/L

Hemoglobin ≥ 100 x 10^9/L

Neurologic Function: Patients must not have had known central nervous system (CNS) involvement (parenchymal or leptomeningeal) with breast cancer

3) Comorbid Illnesses

Any other medical problems (e.g., hypertension, diabetes) were required to be adequately controlled.

4) Other Malignant Diseases

Patients with a history of malignant neoplasm aside from breast cancer were ineligible except for patients treated curatively for basal or squamous cell carcinomas of the skin or carcinoma of the cervix in situ, or who had lived without relapse from any other curatively treated malignancy for more than ten years.

5) Infectious Diseases

Patients who were seropositive for the Human Immunodeficiency Virus (HIV) or who had clinical evidence of AIDS were ineligible.

6) Informed Consent

Patient deemed able to provide informed consent for the study.

7) Response to Induction/Conventional Chemotherapy

Patients with measurable/evaluable disease were required to have at least stable disease (appendix 3) as demonstrated by some objective (i.e., measurable) or subjective (i.e., pain or analgesic requirements) criteria in response to CCT (generally assessed after three to four cycles of therapy). Patients who had undergone resection of all recurrent disease (e.g., chest wall recurrence, breast recurrence) prior to any chemotherapy were staged as having no evidence of disease (NED) and were considered eligible if they did not show evidence of any further recurrent disease during three to four cycles of subsequent chemotherapy.

iv) Between-Group Differences in Eligibility Criteria

The major methodologic difference between selection of treatment groups was that patients in the experimental group had determinations of eligibility made prospectively (i.e., prior to undergoing HDCT/AHPCT) according to individual protocol specifications, whereas the control group, since they were selected retrospectively, had their eligibility determinations made retrospectively (i.e., after patients had received chemotherapy treatment). The most important implications of this difference between selection of study groups are discussed below:

1) Performance Status

Performance status was prospectively measured in all patients in the experimental group. In the control group, because of the retrospective nature of selection, it had to be inferred somewhat arbitrarily based on dictated physician notes for each individual patient, using available comments (e.g., comments made regarding a particular

patient's general physical appearance, findings on physical examination findings, presence of symptoms, physical mobility, etc.) which might imply a poor performance status for that patient.

2) Pulmonary Function Testing

Since pulmonary function testing is not routinely performed in patients with metastatic breast cancer, this data was not available for the control group. As such, the best available surrogate measure of eligibility was to require that control patients have no documented history of significant chronic pulmonary disease (e.g., chronic bronchitis, emphysema, interstitial fibrosis) and no chest radiograph findings which would be considered compatible with such diagnoses. Abnormalities in chest radiographs considered by the attending physician to represent metastatic disease (e.g., lymphangitic carcinomatosis) were not exclusionary.

3) Cardiac Function

At the point of determination of eligibility, most control patients had not had recent radionuclide gated cardiac scans (to determine adequacy of left ventricular function) performed. In most cases, the reason for this was that radionuclide gated cardiac scans had been done at the time of initial diagnosis prior to the patient receiving adjuvant (usually anthracycline based) chemotherapy. It was felt that in these instances two surrogate inclusion criteria would capture an appropriate control group with respect to cardiac function. For patients who had not had radionuclide gated cardiac scans performed or repeated prior to their initial chemotherapy for metastatic disease, one of the following was required for eligibility:

a) The patient must have had a previous normal radionuclide gated cardiac scan or echocardiogram, and the patient must not have received cardiotoxic threshold doses of doxorubicin (≥ 450 mg/m^2) or epirubicin (≥ 800 mg/m^2).

OR

b) The patient must have had no history of cardiac disease and no abnormalities on chest radiograph compatible with cardiac disease (e.g., no radiographic evidence of pulmonary edema or cardiomegaly).

4) Evaluation of CNS Disease

Patients in the UNMC study routinely underwent MRI scanning of the brain to exclude asymptomatic brain metastases. Patients in the Sudbury database were not required to have imaging studies of the head if they were asymptomatic. Similarly, the control group generally would not have undergone any routine imaging studies of the brain, but those who were symptomatic and had brain metastases or meningeal carcinomatosis documented were excluded from the study.

5) HIV Testing

Patients in both experimental databases routinely underwent testing for HIV. The control group did not have this testing performed routinely.

6) Informed Consent

Informed consent was deemed to have been given by all patients in the experimental groups. In the control group, informed consent had to be implied by virtue of the patient accepting chemotherapy. More recently the ORCC has required consent forms

to be completed by all patients undergoing any form of chemotherapy, but this was not in practice during the years defining the study group.

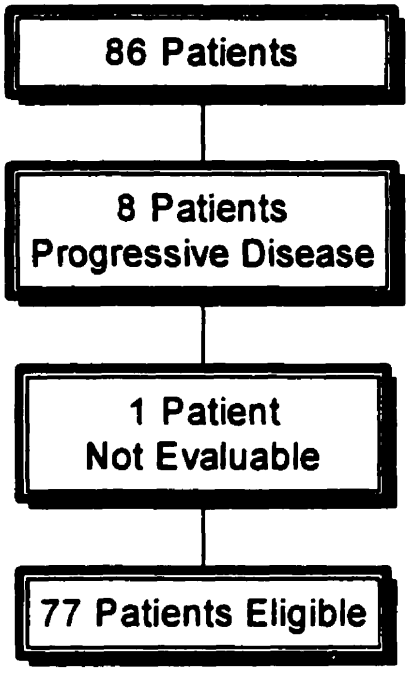
7) Response to Chemotherapy

Protocol requirements generally dictated that patients in the high-dose chemotherapy groups undergo more rigorous (e.g., CT and MRI scans) and more rigorously timed assessments of response to treatment. Control patients generally had response assessments performed with more simple or routine tests such as ultrasound examinations and chest radiographs. Responses in both groups were occasionally determined without strict application of standard response criteria definitions (e.g., when non-measurable sites of disease such as bone were being evaluated), but these determinations are unlikely to have been significantly differentially applied between the two groups.

v) Study Patients

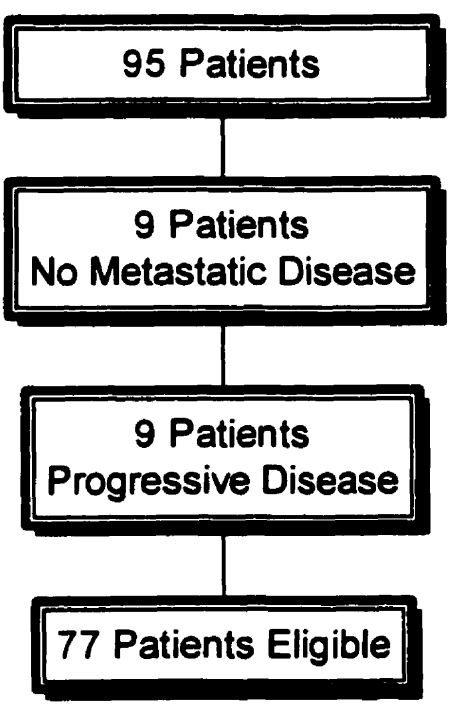
Patients treated between January 1, 1991, and December 31, 1995, formed the study groups. The UNMC dataset contained 86 patients over the pre-determined period of eligibility. After the exclusion of patients who experienced disease progression during induction chemotherapy, and one patient who was not evaluable for response, the UNMC dataset contained 77 eligible patients (Fig 2).

Fig 2. UNMC Patient Eligibility



The NEORCC HDCT/AHPCT dataset initially consisted of 95 patients. After excluding patients who did not have metastatic disease (i.e., who received high-dose therapy for high-risk primary breast cancer), and those who experienced disease progression during induction chemotherapy, the dataset also consisted of 77 eligible patients (Fig 3) for a total of 154 eligible HDCT/AHPCT patients.

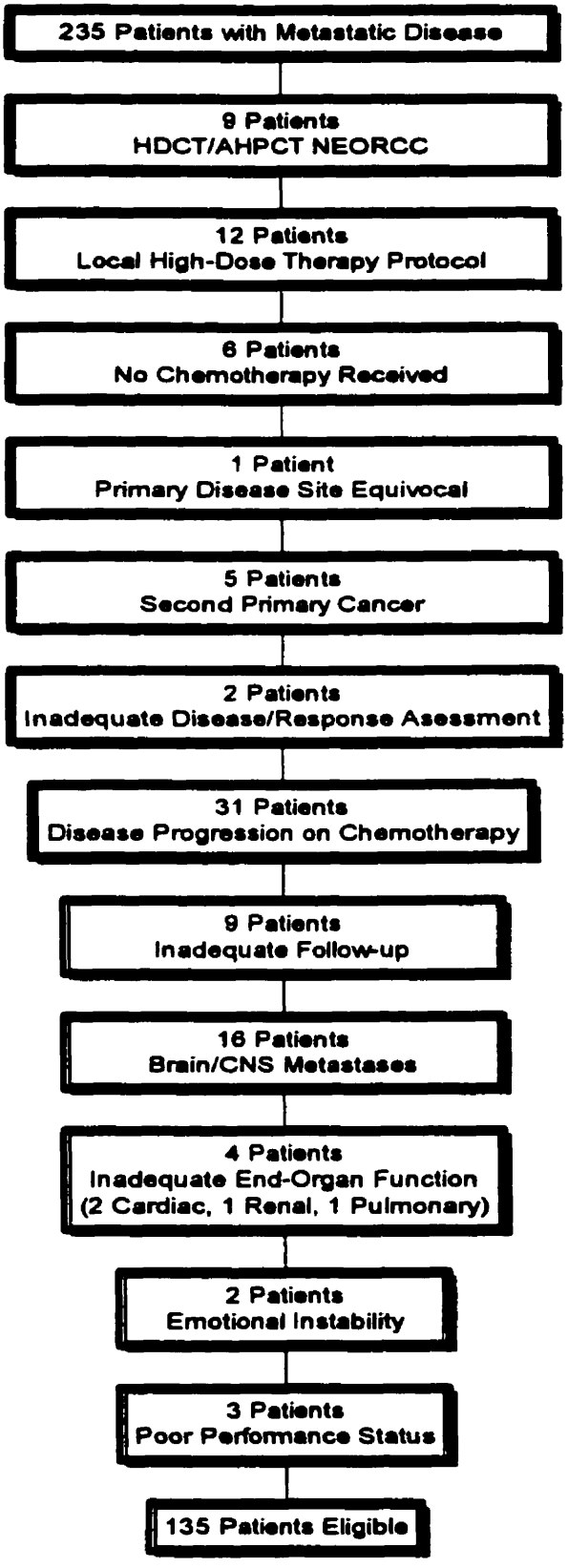
Fig 3. NEORCC Patient Eligibility



The ORCC (control) dataset was compiled from an initial population of 862 patients identified in the OPIS search. However, because of standard coding practices in OPIS, the search algorithm unexpectedly retrieved a number of patients with axillary node positive disease (considered by OPIS to be a site of metastases) at diagnosis but no subsequent distant metastases. Since these patients did not have distant metastatic disease they had to be manually excluded from the study. However there was no way to clearly separate these patients out by the OPIS search algorithm. The selection of controls therefore had to be performed by random review of this population of 862 patients, manually excluding the above described patients (who had no distant metastases) as they were detected. Time limitations permitted a random review of 400 charts from among the initial population of 862, and from those 400 charts, 235 patients were identified as having metastatic disease (Fig 4).

Of these 235 potentially eligible control patients, nine had received HDCT/AHPCT at the NEORCC, and 12 had received high-dose chemotherapy (without progenitor cell infusion) as part of a local study protocol, and were therefore excluded from the CCT dataset. Six patients were excluded on the basis of not having received any chemotherapy for metastatic disease, 31 patients were excluded for non-response to CCT, and 16 patients were excluded for brain or CNS metastases. Twenty six additional patients (for a total of 79 patients) were deemed ineligible by the selection criteria established for the control group. The remaining 135 eligible patients made up the CCT group (Fig 4).

Fig 4. ORCC Patient Eligibility



The exact number of patients excluded from receiving high-dose chemotherapy at UNMC and NEORCC for all of the above selection criteria was not accurately known for two principal reasons. First, this type of data was not strictly required to be recorded by either transplant centre during trial enrollment. Second, in many instances, decisions on the part of primary care oncologists may have prevented patients who were obviously not candidates for HDCT/AHPCT (e.g., patients with brain metastases) from being referred to the centres for evaluation. However, for those patients who did receive evaluations for HDCT/AHPCT at Omaha, it was known that two patients were excluded for cardiac dysfunction, one for pulmonary dysfunction, and six for asymptomatic brain metastases found on CT/MRI scanning. The majority of patients excluded for other reasons were for progression during induction therapy. For patients who received evaluation in Sudbury, it was known that one patient was refused HDCT/AHPCT for poor performance status, two for cardiac dysfunction, two for lack of ability to give informed consent, thirteen for progression during induction chemotherapy, and that one patient decided against HDCT/AHPCT after achieving a complete response to chemotherapy.

vi) Treatment Protocols

1) UNMC Protocol (HDCT/AHPCT)

Patients were referred to UNMC from other cancer centres (both local, and out of state) in the United States for consideration of HDCT/AHPCT. Subsequent to confirmation of eligibility, patients generally received three to four cycles of initial CCT (termed induction chemotherapy) followed by an assessment of response. Patients with at least stable disease, or more often a confirmed response to CCT went on to receive

high-dose therapy. Four patients with no clinically measurable/evaluable metastatic disease (e.g., where the only site had been resected surgically, or where only bone marrow micrometastases were detected) did not undergo any induction chemotherapy prior to receiving high dose therapy. Patients underwent progenitor cell collection using either unstimulated bone marrow, growth factor mobilized peripheral blood, or occasionally both as the source(s) of cells. The selection of progenitor cell source was largely dependent upon the chronologic time of transplant (use of peripheral blood underwent a slow temporal evolution to overtake bone marrow as the more commonly accepted source of progenitor cells). Prior to 1993, bone marrow was used more commonly, although not exclusively as the source of cells. From April 1993 onward, all patients received peripheral blood progenitor cells as the source of hematopoietic rescue. Patients were required to have had collected at least 2.0×10^8 mononuclear cells/kg by bone marrow harvest, or 6.5×10^8 mononuclear cells/kg by peripheral blood apheresis prior to proceeding with HDCT/AHPCT.

The high-dose chemotherapy regimen was as follows:

Cyclophosphamide 1.5 g/m^2/day intravenously as a continuous infusion for 4 days.
Thiotepa 150 mg/m^2 /day intravenously as a continuous infusion for 4 days.
Hydoxyurea 1.5 g/m^2 orally q 6 hours for 12 doses.
Progenitor cell infusion 72 hours after the last doses of thiotepa and cyclophosphamide.

Following progenitor cell infusion patients received empiric supportive care according to institutional standards to maintain adequate nutritional (enteral or parenteral nutrition), hematologic (prophylactic red cell and platelet transfusions to maintain adequate hemoglobin and platelet levels), and antimicrobial (empiric antibiotics for fever and

neutropenia, adjusted appropriately based on culture results) support. Other supportive care measures (e.g., narcotics for mouth pain or mucositis, anti-diarrheal medication, intravenous fluids for dehydration) were given as required.

Following recovery, patients remained under the care of the transplant centre until hematologically and nutritionally independent, at which time they were discharged from the cancer centre's care. Complete re-staging was performed at UNMC at 100 days post transplant, and patients were then returned to the full time care of their referring medical oncologist. Continued follow-up for progression/relapse and survival information was obtained by interval contact with referring physicians. Patients who had not had an event (relapse/progression or death) at last contact were censored for that outcome in the database. The database was last updated June 1996.

2) NEORCC Protocol (HDCT/AHPCT)

Patients were referred to Sudbury Ontario for HDCT/AHPCT from local cancer physicians and from other provincial regional cancer centres. Subsequent to confirmation of eligibility criteria, patients with any evaluable/measurable disease generally received three cycles of induction chemotherapy followed by an assessment of response. Patients with no measurable/evaluable metastatic disease (e.g., where the only site had been resected surgically or treated with radiotherapy) still underwent induction chemotherapy, and went on to HDCT/AHPCT if no new sites of disease appeared during the period of induction chemotherapy. After confirmation of a response to CCT (at least symptomatic if no obvious change on imaging studies, e.g., bone scans or plain radiographs of bone), all patients underwent peripheral blood progenitor cell collection (generally using one cycle of FAC chemotherapy (5-fluorouracil, doxorubicin, cyclophosphamide) followed by hematopoietic growth factor

mobilization with granulocyte colony-stimulating factor (G-CSF). Bone marrow was not used as a source of hematopoietic progenitor cells for any patients in the Sudbury database. Patients were required to have had collected at least 2.0×10^8 mononuclear cells/kg, or 2.0×10^6 CD34 positive cells/kg by peripheral blood apheresis prior to proceeding with HDCT/AHPCT.

Sudbury patients were treated on a variety of high-dose chemotherapy protocols over the study period:

Regimen 1:

Cyclophopsphamide 3 g/m^2/day intravenously for 2 days.

Mitoxantrone 23 mg/m^2/day intravenously for 3 days.

Vinblastine 12 mg/m^2 intravenously as continuous infusion over 5 days.

Regimen 2:

Cyclophopsphamide 3 g/m^2/day intravenously for 2 days.

Mitoxantrone 23 mg/m^2/day intravenously for 3 days.

Carboplatin 800 mg/m^2 intravenously for 1 day.

Regimen 3:

Cyclophopsphamide 3 g/m^2/day intravenously for 2 days.

Mitoxantrone 23 mg/m^2/day intravenously for 3 days.

Paclitaxel 250-450 mg/m^2 intravenously for 1 day (phase I/II study).

Regimen 4:

Cyclophopsphamide 3 g/m^2/day intravenously for 2 days.

Thiotepa 500 mg/m^2 intravenously for 1 day.

Carboplatin 800 mg/m^2 intravenously for 1 day.

Progenitor cell infusion 48-72 hours post chemotherapy, depending on chemotherapy regimen.

Progenitor cell infusion was followed by empiric supportive care to maintain adequate nutritional (enteral or parenteral nutrition), hematologic (prophylactic red cell and platelet transfusions to maintain adequate hemoglobin and platelet levels), and antimicrobial (empiric antibiotics for fever, adjusted appropriately based on culture results) support. Other supportive care (e.g., narcotics for mouth pain or mucositis, anti-diarrheal medication, intravenous fluids for dehydration) were given as required. All supportive care procedures followed institutionally accepted standards.

Following recovery, patients remained under the care of the transplant centre until hematologically and nutritionally independent, at which time referred patients were discharged from the cancer centre's care, and returned to the full time care of their referring medical oncologist. Continued follow-up for relapse and survival information was obtained by interval contact with referring physicians. Patients who had not relapsed/progressed at last contact were censored for this outcome, as were patients who were still alive at last contact (last updated June 1997).

3) ORCC Protocols (CCT)

Patients were generally referred to the ORCC through local referral channels (primary care physicians, general internists, general surgeons, and subspecialists). The vast majority of patients had been referred at initial diagnosis, prior to the development of any metastatic disease. Subsequent to confirmation of eligibility criteria, patients with any evaluable/measurable disease received CCT with an assessment of response

generally performed after three to four cycles of therapy according to the treating physicians usual standard of care. Patients with no measurable/evaluable metastatic disease (e.g., where the only site had been resected surgically, or treated with radiotherapy) who underwent CCT after resection or radiotherapy were considered "responders" if no new disease appeared over the first four cycles of chemotherapy.

The approach to chemotherapy was at the discretion of the treating physicians. However, a common approach would often be as follows:

For patients who had received prior adjuvant chemotherapy, the following four regimens and general dosage accounted for the vast majority of chemotherapy:

Oral CMF: (repeated at four week intervals):

Cyclophopsphamide 100mg/m^2 orally day 1 to day 14.

Methotrexate 40 mg/m^2 intravenously day 1, day 8.

5-Fluorouracil 600 mg/m^2 intravenously day 1, day 8.

Intravenous CMF (repeated at three week intervals):

Cyclophopsphamide 600mg/m^2 intravenously day 1.

Methotrexate 40 mg/m^2/day intravenously day 1.

5-Fluorouracil 600 mg/m^2 intravenously day 1.

Intravenous FAC: (repeated at three week intervals):

Cyclophopsphamide 500mg/m^2 intravenously day 1.

Doxorubicin 50 mg/m^2 intravenously day 1.

5-Fluorouracil 500 mg/m^2 intravenously day 1.

Intravenous FEC (repeated at three week intervals):

Cyclophopsphamide 500mg/m^2 intravenously day 1.

Epirubicin 50 mg/m^2 intravenously day 1.

5-Fluorouracil 500 mg/m^2 intravenously day 1.

If a patient had not received prior adjuvant therapy, the first line metastatic treatment was most often anthracycline based (FEC or FAC), although a few patients were treated with CMF.

c) For patients who had received anthracycline based therapy in the adjuvant setting the first line metastatic chemotherapy was most often single agent paclitaxel (125-175 mg/m^2) intravenously or docetaxel (100 mg/m^2) intravenously every three weeks.

Other relatively common chemotherapy regimens included vinorelbine, 5-fluorouracil/folinic acid, 5-fluorouracil/folinic acid/mitoxantrone, etoposide/cisplatin, and mitomycin-C/vinblastine, all given in conventional dose ranges and schedules. The duration of chemotherapy (number of cycles) was at the treating physician's discretion (median = 6), and generally depended on tolerance of therapy as well as ongoing response to therapy.

Patients received empiric supportive care as required (e.g., antibiotics for infection, red cell and platelet transfusions as required to maintain adequate hemoglobin and platelet levels) at the treating physician's discretion. Other supportive care (e.g., narcotics for pain, radiotherapy for nodal areas or painful lesions) was also used as per usual standards of care.

For the purposes of data collection/evaluation, CCT patients were followed using fully dictated/typed progress notes, bloodwork, and the results of radiologic investigations, noting the first appearance of either progression or recurrence as indicated by the treating physician or by imaging studies. Patients were followed through until death or last follow-up, and were censored for the purposes of analysis when the event of interest had not occurred at last follow-up, with data having been followed through until August 1997.

vii) Data Collection/Retrieval/Compilation

1) UNMC Database

Data in the UNMC database was collected by a single clinical nurse transplant coordinator working with Drs. Reed and Tarantolo. Data was gathered for each individual patient using source documentation (pathology reports, radiographs and radiograph reports), historical/personal/health information supplied by the patient and referring physicians, and by telephone contact subsequent to discharge from UNMC as required. For each patient, the data was compiled into a separate UNMC patient research chart, and was subsequently entered into the UNMC database by specific trained data management personnel. Data was coded as per institutional specifications and entered/managed using SAS software.

Data from the UNMC database was received as an ASCII text file without delimiters, and converted to Microsoft Excel format for all further manipulation. Importing this data into Excel involved setting up multiple column delimiters for multiple variables and multiple pages of data. This process required a substantial degree of editing. As this could have increased the probability of error, each data value for each patient/variable

was checked against an original unmodified printed version of the database to ensure accuracy of the data importing/editing procedure. Existing data was then reviewed and summarized to create new variables (generally categorical) to include data which had been entered in text formats (e.g., prior therapy, sites of metastatic disease). All new variables created in this format were twice verified for accuracy of entry.

2) NEORCC Database

Data from the NEORCC database was collected by individuals working in the NEORCC clinical trials department. Again data for each individual patient was gathered using source documentation, information supplied by patients and referring physicians, and subsequent telephone contact as required. For each patient, the data was compiled into a separate patient research chart, and trained data management personnel subsequently entered this data into the NEORCC database. Data was coded as per institutional specifications and stored on the Medlog database system.

Data from the NEORCC database was received from Medlog as a delimited ASCII text file, which meant that no significant column/variable editing was required in converting the file to the Microsoft Excel format. Again all existing data was reviewed and summarized to create new variables to include data that had been received in text formats. All new variables created in this format were twice verified for accuracy of entry.

3) ORCC Database

Data in the ORCC database was collected by the master's student. Some initial chart screening was performed by a summer student working in the ORCC (see acknowledgments). After random selection of an ORCC patient chart (see study design/methodology), the chart was reviewed for eligibility criteria. Charts meeting eligibility requirements were then checked for completeness of information (e.g., for adequacy and duration of follow-up, dates of progression, death etc). Data from eligible patient charts containing complete follow-up information was then recorded on separate individual patient data sheets (appendix 7) containing as much information as possible that was common to the other two databases.

This data was then entered into Microsoft Excel in generally in numerical (continuous and categorical) format. Data entered was verified by double checking each complete patient record after entry.

4) Final Common Database

Each institution's database was generated on a separate page within a Microsoft Excel file, and all three were then re-coded where required and combined onto a single page to produce a final common variable format. All continuous variables were captured and/or calculated (e.g., outcome times, patient ages) in their original continuous form, and were not altered in any way. All durations of time (age at diagnosis, age at chemotherapy, disease-free interval, time to failure, and overall survival) were calculated using calendar dates, with calculations having been rounded off to the nearest tenth of a month. Dates were entered in the format day/month/year, and all date math was performed within the Excel spreadsheet.

viii) Variables Contained in the Combined Database

The final combined database contained data which was available and common to patients from all three datasets. The complete variable list and descriptors (with codes) for each variable is contained in appendix 8.

ix) Outcome Variable Definitions and Time Points for Analyses

Overall survival was measured from the time of disease recurrence until the occurrence of death from any cause. Patients not known to have died were censored for this outcome at last known follow-up.

Time to failure was measured from the time of initiation of chemotherapy until the occurrence of any one of the following events:

1. Recurrence of disease after having achieved a complete remission.
2. Recurrence of disease for patients who had no evidence of disease (NED) at the initiation of chemotherapy.
3. Progression of disease after achieving a partial remission.
4. Death from any cause.

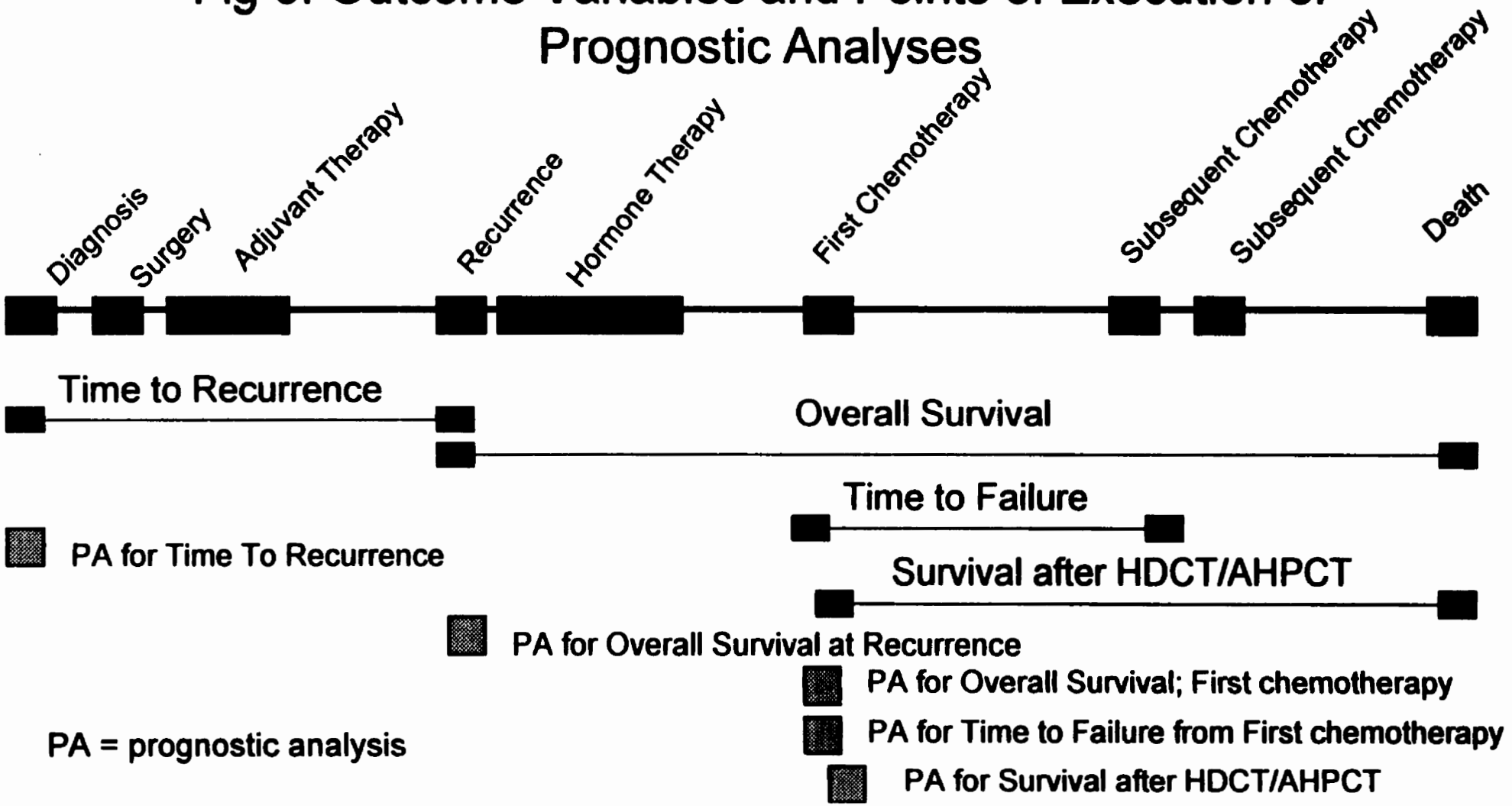
Patients not known to have had any of these events were censored for this outcome at the point of last known follow-up.

Survival after HDCT/AHPCT was measured from the point of initiation of HDCT/AHPCT until the occurrence of death from any cause. Patients not known to have died were censored for this outcome at last known follow-up.

Figure 5 demonstrates a hypothetical time line of typical disease and therapy related events for a patient with breast cancer from diagnosis through recurrence and death. Included in the figure are graphic illustrations of outcome variables and points of execution of various prognostic analyses (see section V., statistical analysis).

The prognostic analyses for overall survival and time to failure at the point of initiation of chemotherapy utilized sites of disease at the initiation of chemotherapy as well as prior treatment related information such as the use of prior adjuvant chemotherapy , prior adjuvant hormonal therapy, and prior metastatic hormonal therapy.

Fig 5. Outcome Variables and Points of Execution of Prognostic Analyses



V. Statistical Analysis

i) Descriptive Statistics

Categorical data was captured and coded using numeric values, with appropriate text/character references for each variable being entered simultaneously into the various statistical software packages which were used to collect and analyze the data. Within-group descriptive statistics (e.g., frequency distributions, and median values) were generated using standard descriptive statistical algorithms.

ii) Inferential Statistics

1) Between-Group Univariate Comparisons: Baseline Variables

Univariate comparisons of baseline variables were performed using chi-Square analyses in the case of categorical variables, and the Student's t-test or Mann-Whitney U-test as appropriate for continuous and ordinal variables.

2) Between-Group Univariate Comparisons: Outcome Variables

Time to event data (overall survival, time to failure) was analyzed using the product-limit method of Kaplan and Meier.[137] For between-group comparisons of time to event endpoints, univariate comparisons were carried out using logrank tests on Kaplan-Meier plots.

3) Between-Group Multivariate Comparisons: Outcome Variables

Time to event data was analyzed using the Cox proportional hazards model[61] with forward stepwise regression to adjust for the effects of multiple confounding variables.

Assumptions underlying the Cox Proportional Hazards Model

The Cox proportional hazards model is used when time-to-response data is influenced by the presence of other variables (covariates) and contains a number of underlying assumptions:

The model assumes that death rates may be modeled as log-linear functions of the aforementioned covariates. The hazard function is represented by the following equation:

$$h(t:z) = h_0(t)\exp(B_1z_1 + B_2z_2 + B_3z_3 + B_nz_n)$$

A hazard is defined as the instantaneous rate of occurrence of an event at time "t" given that the event has not yet occurred up to time "t". The expression $h_0(t)$ represents the hazard function for an individual in whom the value of the covariate z is 0. B_1 represents the regression coefficient for the covariate z_1. The first assumption of the Cox proportional hazards model is that the relationship between the underlying hazard function and the effect of a covariate is multiplicative (log-linear). The second assumption is that the effect of a covariate is not time dependent, and remains proportional over time.

The proportionality assumption can be tested by plotting the log(-log(S(t))) versus log (t for each level of covariates. If the proportionality assumption is valid, the lines should be parallel. For this thesis, the proportionality assumption was not tested for each analysis; rather, one log minus log plot was generated for estrogen receptor positivity and overall survival (appendix 9). With these underlying assumptions, the following major analyses were carried out (see also Fig 5, p 49):

a) Analysis of predictors of time to initial recurrence (univariate and multivariate). This analysis was performed largely to help confirm the validity of the database by comparing the results to previously reported literature.

b) Analysis of predictors of overall survival (univariate and multivariate) from the initial point of metastatic disease. This analysis was also performed largely to help confirm the validity of the database by comparing the results to previously reported literature.

c) Analysis of predictors of overall survival (univariate and multivariate) at the time of initiation of chemotherapy, including the measurement of the independent effect of treatment (CCT versus HDCT/AHPCT) on overall survival.

d) Analysis of predictors of time to failure (univariate and multivariate) at the time of initiation of chemotherapy, with and without the independent effect of treatment (CCT versus HDCT/AHPCT) on the probability of failure over time.

e) Analysis of predictors of survival after transplantation (univariate and multivariate), both at baseline, and including the effect of several potentially important transplant related variables (e.g., treatment centre, chemotherapy regimen, and source of progenitor cells).

All univariate statistics reported in the multivariate analysis tables (tables 6, 9, 10, 11, 12, 14, 15) were generated by using the unadjusted P values given by the model prior to the occurrence of any stepping. Conceptually this would be equivalent to running a univariate Cox proportional hazards analysis for each variable.

iii) Sample Size Calculation

Assuming a median survival of two years for conventionally treated patients, the ability to show a 20% absolute increase in survival at two years for patients treated with high-dose therapy (allowing for a type I error of 0.05 and a type II error of 0.10) would require a total sample size of approximately 280 patients, or 140 patients per group (based on a two group comparison). Although 150 patients in each group were planned for the study and analysis, the available number of control patients fell slightly short of this number.

iv) Statistical Packages/Software

Statistical packages and software utilized included Microsoft Office (random number generation, spreadsheet creation, datemath), BMDPNS for PC version 1.0 (univariate descriptive and inferential statistics), BMDP Classic (life table analysis calculations, Cox proportional hazards regression analyses), Graphpad Prism (graphical renderings of Kaplan-Meier curves), and Power (sample size calculation).

VI. Results

i) Baseline Characteristics of Groups at Diagnosis

Table 4 reveals the baseline characteristics of the patients from UNMC (HDCT/AHPCT) and NEORCC (HDCT/AHPCT) with respect to primary disease characteristics (i.e., at initial diagnosis). There were no significant baseline differences between the two groups of patients with the exception of prior adjuvant therapy. Fewer NEORCC patients had received adjuvant chemotherapy (P=0.027), but more had received adjuvant hormone therapy (P<0.001) in comparison with the UNMC patients. Only the latter observation would be considered statistically significant after adjustment for multiple testing.

[redacted]

Variable	Omaha (HDCT/AHPCT)	Sudbury (HDCT/AHPCT)	P
N	77	77	NA
Median age at Diagnosis (IQR)	40 (35-45)	43 (38-47)	0.101
Breast N (%)			0.307
Left	32 (42)	37 (49)	
Right	44 (57)	35 (46)	
Bilateral	1 (1)	3 (4)	
Initial Stage N (%)			0.188
I	15 (19)	10 (13)	
IIA	21 (27)	23 (30)	
IIB	13 (17)	21 (27)	
IIIA	9 (15)	5 (6)	
IIIB	7 (9)	2 (3)	
IV	12 (16)	16 (21)	
Number of involved nodes (%)			0.409
0	28 (38)	26 (38)	
1-3	21 (29)	14 (24)	
4-9	11 (15)	16 (19)	
≥10	13 (18)	15 (19)	
Median	1	3	
Estrogen Receptors N (%)			0.434
Negative	37 (48)	30 (39)	
Positive	38 (49)	40 (52)	
Unknown	2 (3)	7 (9)	
Progesterone Receptors N(%)			0.656
Negative	30 (39)	34 (44)	
Positive	37 (48)	36 (47)	
Unknown	10 (13)	7 (9)	
Adjuvant Chemotherapy N (%)			0.027
None	23 (30)	38 (49)	
CMF based	30 (39)	26 (34)	
Anthracycline based	24 (31)	13 (17)	
Adjuvant Hormone Therapy N (%)			< 0.001
No	68 (88)	49 (64)	
Yes	9 (12)	28 (36)	

HDCT/AHPCT=high-dose chemotherapy/autologous hematopoietic progenitor cell transplantation; CCT=conventional chemotherapy; IQR=inter-quartile range; NA=not applicable

Table 5 combines the two experimental groups into one HDCT/AHPCT group and displays the baseline characteristics of the experimental and control groups with respect to primary disease. There were some notable differences between the two groups with respect to primary disease characteristics. The HDCT/AHPCT in general was slightly younger at diagnosis, and contained a slightly greater proportion of patients with stage III disease. Patients in the HDCT/AHPCT group were also more commonly estrogen and progesterone receptor negative but despite this tended to have received more adjuvant hormonal therapy in comparison with the CCT group. Again, after adjustment for multiple testing, only age at diagnosis and the proportion of patients with estrogen receptor positivity would be considered statistically significant.

Variable	HDCT/AHPCT	CCT	P
N	154	135	NA
Median age at Diagnosis (IQR)	41 (37-46)	44 (40-48)	0.006
Breast N (%)			0.230
Left	69 (45)	72 (53)	
Right	79 (52)	62 (46)	
Bilateral	4 (3)	1 (1)	
Initial Stage N (%)			0.050
I	25 (16)	17 (13)	
IIA	44 (29)	42 (31)	
IIB	34 (22)	40 (30)	
IIIA	14 (9)	2 (1)	
IIIB	9 (6)	12 (9)	
IV	28 (18)	22 (16)	
Number of involved nodes (%)			0.017
0	54 (36)	42 (34)	
1-3	35 (24)	38 (31)	
4-9	27 (19)	33 (27)	
≥10	28 (19)	9 (7)	
Median	2	2	
Estrogen Receptors N (%)			0.003
Negative	67 (43)	34 (25)	
Positive	78 (51)	85 (63)	
Unknown	9 (6)	16 (12)	
Progesterone Receptors N(%)			0.053
Negative	64 (42)	41 (30)	
Positive	73 (47)	77 (57)	
Unknown	17 (11)	17 (13)	
Adjuvant Chemotherapy N (%)			0.066
None	61 (39)	64 (47)	
CMF based	56 (36)	32 (24)	
Anthracycline based	37 (24)	39 (29)	
Adjuvant Hormone Therapy N (%)			0.013
No	117 (76)	118 (87)	
Yes	37 (24)	17 (13)	

HDCT/AHPCT=high-dose chemotherapy/autologous hematopoietic progenitor cell transplantation; CCT=conventional chemotherapy; IQR=inter-quartile range; NA=not applicable

ii) Prognostic Factors for Time to Recurrence (Disease-Free Interval)

As the recurrence of breast cancer can be predicted by various prognostic factors previously cited in the literature,[19,26,27,30-32] an analysis of prognostic factors for time to recurrence was performed using 238 patients for whom complete baseline data was available (table 6). Multivariate analysis was performed using the Cox proportional hazards model[61] with forward stepwise regression. Since this analysis was designed to identify prognostic factors for a shorter time to recurrence, disease-free interval was used as the time dependent outcome variable. As all patients by definition had experienced an initial recurrence of disease, no patients were censored for this analysis.

Table 6: Prognostic Factors for Time to Recurrence

Variable	P Value (Univariate)	P Value (Multivariate)	HR (Multivariate)	SE	95% CI
Age at Diagnosis	**0.003**	**0.066**	**1.02**	**0.010**	**1.00-1.04**
Number of Positive Axillary Nodes	0.009	0.191	NC	NC	NC
Initial Clinical Stage	**< 0.001**	**< 0.001**	**1.58**	**0.058**	**1.47-1.69**
Estrogen Receptor Positivity	< 0.001	0.325	NC	NC	NC
Progesterone Receptor Positivity	**0.014**	**0.047**	**0.77**	**0.134**	**0.61-1.03**

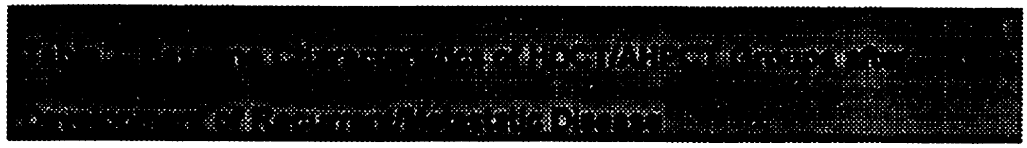
HR = hazard ratio; SE = standard error; 95% CI = 95% confidence interval;
NC=not calculated by BMDP software

Exploratory analyses for the presence of interaction terms were performed on variables with an independent effect on time to recurrence. These analyses revealed no

significant interaction between age and hormone receptor status, stage and hormone receptor status, or between age and advancing stage.

iii) Baseline Characteristics of Groups at Recurrence

Tables 7 and 8 display the characteristics of the study groups after the development of recurrent/metastatic disease. Table 7 reveals the characteristics of the patients from UNMC (HDCT/AHPCT) and NEORCC (HDCT/AHPCT). Although there appear to be differences between the two groups with respect to number of metastatic hormonal therapies received prior to beginning chemotherapy, and the proportion of patients with lung and pleural involvement, none of these differences are considered statistically significant after adjusting for multiple testing.



Variable	Omaha (HDCT/AHPCT)	Sudbury (HDCT/AHPCT)	P
N	77	77	NA
Median Disease-Free Interval in Months (IQR)	22.1 (10.5-35.7)	19.1 2.3-38.7	0.668
Median Age at Beginning of Chemotherapy (IQR)	42 (38-47)	45 (40-50)	0.115
Number of Metastatic Hormones N (%)			0.009
None	50 (65)	35 (45)	
One	19 (25)	17 (22)	
Two	6 (8)	19 (25)	
Three	2 (2)	6 (8)	
Four	0 (0)	0 (0)	
Median	0	1	
Number of Disease Sites N (%)			0.580
None (NED)	2 (3)	2 (3)	
One	43 (56)	46 (60)	
Two	22 (29)	24 (31)	
Three	7 (9)	3 (4)	
Four	3 (4)	1 (1)	
Five	0 (0)	1 (1)	
Median	1	1	
Visceral Disease N (%)			0.098
Yes	25 (32)	35 (45)	
No	52 (68)	42 (55)	
Bone N (%)	40 (52)	39 (51)	0.871
Liver N (%)	14 (18)	13 (16)	0.832
Lung N (%)	16 (20)	28 (36)	0.032
Pleural N (%)	5 (6)	0 (0)	0.023
Skin N (%)	6 (7)	4 (0)	0.513
Nodal N (%)	20 (26)	20 (26)	1.000
Locoregional Disease N (%)	14 (18)	10 (13)	0.374
Response to Chemotherapy N (%)			0.104
Stable Disease	10 (13)	14 (18)	
Partial Response	46 (60)	54 (70)	
Complete Response	18 (23)	8 (10)	
No Evidence of Disease	3 (4)	1 (1)	

HDCT/AHPCT = high-dose chemotherapy/autologous hematopoietic progenitor cell transplantation; CCT = conventional chemotherapy; NA=not applicable;
IQR = inter-quartile range

Table 8 combines the two experimental groups into one HDCT/AHPCT group for comparison with the CCT group. After adjusting for the effect of multiple testing, some differences between the two groups continued to be evident. The HDCT/AHPCT group on average were younger at the point of beginning chemotherapy, had received fewer hormonal therapies for metastatic disease, had fewer sites of disease, and contained a smaller proportion of patients with liver metastases and with locoregional disease.

Variable	HDCT/AHPCT	CCT	P
N	154	135	NA
Median Disease-Free Interval (Months) (IQR)	19.6 (8.9-36.0)	22.7 (10.7-36.7)	0.060
Median age at Beginning of Chemotherapy (IQR)	44 (39-48)	47 (42-50)	<0.001
Number of Metastatic Hormones N (%)			<0.001
None	85 (56)	30 (22)	
One	36 (23)	46 (34)	
Two	25 (16)	39 (29)	
Three	8 (5)	17 (13)	
Four	0 (0)	3 (2)	
Median	0	1	
Number of Disease Sites N (%)			<0.001
None (NED)	4 (3)	11 (8)	
One	89 (57)	37 (27)	
Two	46 (30)	44 (33)	
Three	10 (6)	25 (19)	
Four	4 (3)	14 (10)	
Five	1 (1)	4 (3)	
Median	1	2	
Visceral Disease N (%)			0.490
Yes	60 (39)	58 (43)	
No	94 (61)	77 (57)	
Bone N (%)	79 (51)	69 (51)	0.975
Liver N (%)	27 (17)	45 (33)	0.002
Lung N (%)	44 (29)	21 (16)	0.008
Pleural N (%)	5 (3)	16 (11)	0.005
Skin N (%)	10 (6)	8 (5)	0.842
Nodal N (%)	40 (26)	44 (33)	0.216
Locoregional Disease N (%)	24 (27)	62 (72)	<0.001
Response to Chemotherapy N (%)			0.008
Stable Disease	24 (16)	15 (11)	
Partial Response	100 (65)	92 (68)	
Complete Response	26 (17)	13 (9)	
No Evidence of Disease	4 (3)	15 (11)	

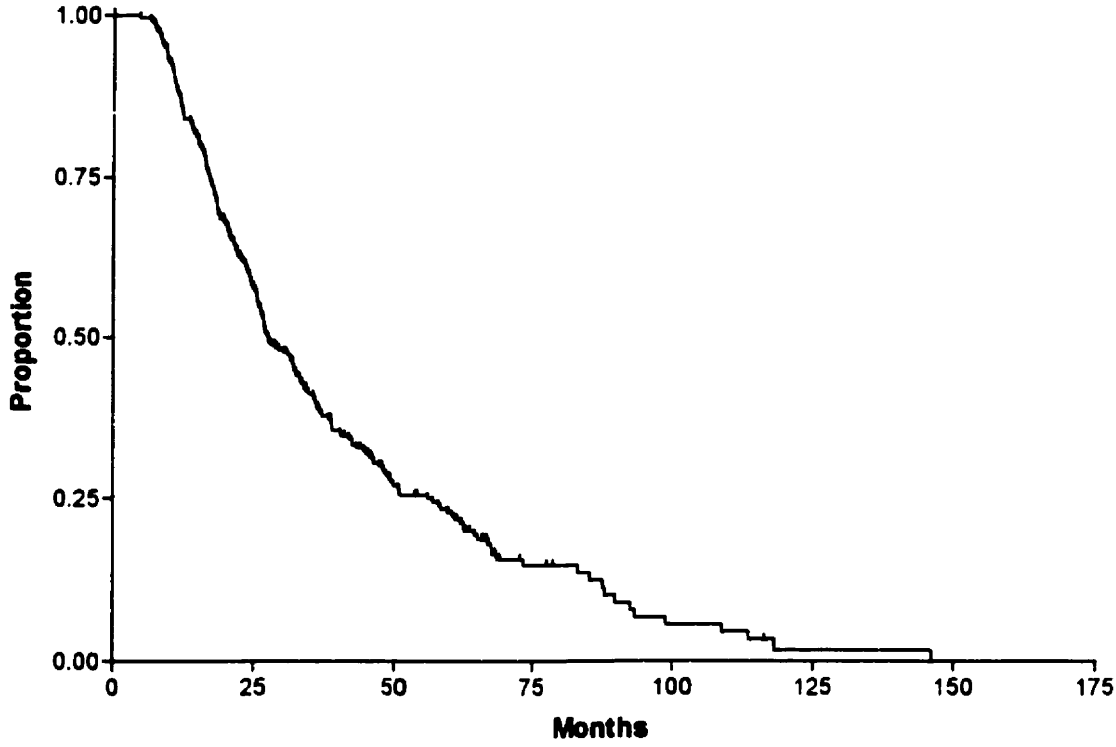
HDCT/AHPCT = high-dose chemotherapy/autologous hematopoietic progenitor cell transplantation; CCT = conventional chemotherapy; NA=not applicable;
IQR = inter-quartile range

iv) Overall Survival Outcomes

1. Overall Survival; All Patients

The median survival of all patients after the development of recurrent/metastatic disease (Fig 6) was 27.4 months (95% confidence interval [CI]=25.6-33.1 months).

Fig 6. Overall Survival from Metastases; All Patients



2. Prognostic Factors for Overall Survival at Development of Metastatic Disease

Prognostic factors that were predictive of a longer overall survival at the initial point of metastatic disease are provided in table 9. This analysis therefore excluded all variables related to treatment of metastatic disease, and sites of metastatic disease represent sites of disease at the initial appearance of metastatic disease. This analysis was conducted on 213 patients (Ottawa and Omaha patients), as the initial sites of metastatic disease were not available in the Sudbury database. Multivariate analysis using the Cox proportional hazards model[61] with forward stepwise regression revealed that progesterone receptor positivity, the presence of bone metastases, and the presence of locoregional disease were all independent predictors of longer survival, whereas prior adjuvant chemotherapy, prior adjuvant hormone therapy, liver metastases, and an increasing number of sites of disease were all independent predictors of a shorter survival.

[redacted]

Variable	P Value (Univariate)	P Value (Multivariate)	HR (Multivariate)	SE	95% CI
Initial Stage	0.021	0.551	NC	NC	NC
Estrogen Receptor Positivity	0.007	0.189	NC	NC	NC
Progesterone Receptor Positivity	0.001	0.011	0.62	0.184	0.26-0.98
Prior Adjuvant Chemotherapy	< 0.001	<0.001	2.34	0.204	1.94-2.74
Prior Adjuvant Hormone Therapy	0.116	0.002	2.33	0.259	1.83-2.83
Disease-Free Interval	0.011	< 0.001	0.98	0.006	0.97-.099
Bone Metastases	0.101	0.013	0.59	0.214	0.17-1.01
Liver Metastases	<0.001	0.056	1.68	0.266	1.09-2.12
Pleural Metastases	0.006	0.342	NC	NC	NC
Nodal Metastases	0.003	0.981	NC	NC	NC
Locoregional Disease	<0.001	< 0.001	0.45	0.217	0.02-0.88
Number of Disease sites	<0.001	<0.001	2.06	0.141	1.78-2.34
Visceral Disease	< 0.001	0.701	NC	NC	NC

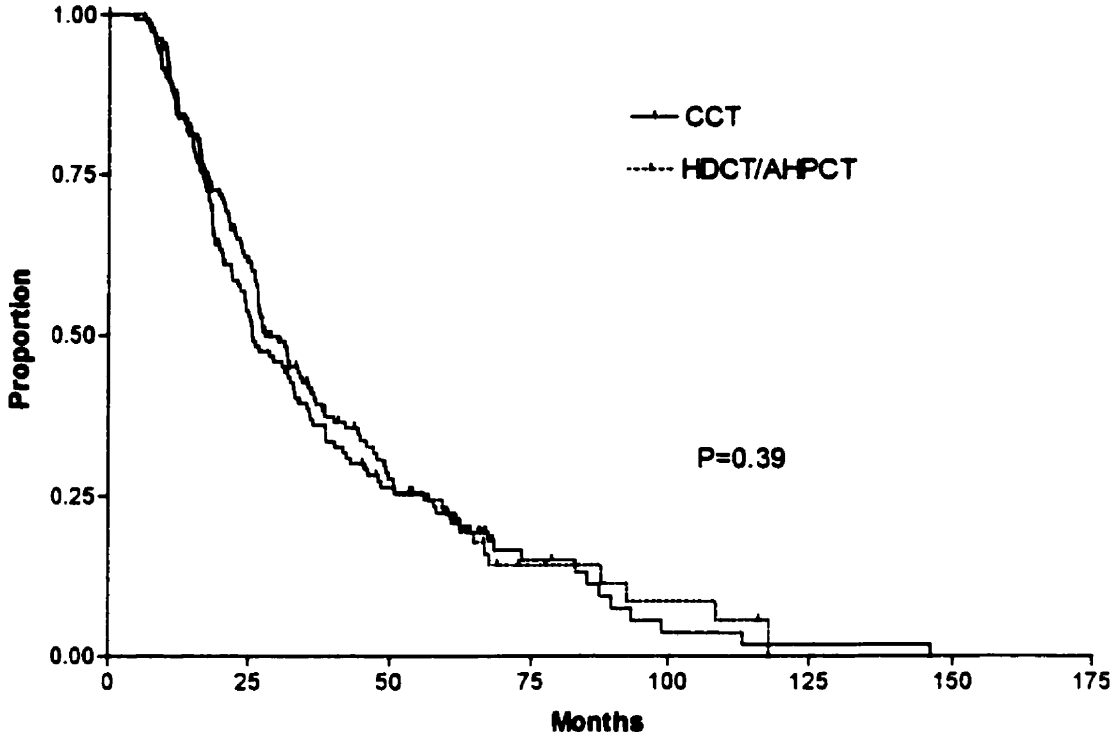
HR = hazard ratio; SE = standard error; 95% CI = 95% confidence interval;
NC=not calculated by BMDP software

Exploratory analyses were performed on variables with known or hypothesized interactions. The results of such analyses revealed the presence of an interaction between initial stage and adjuvant chemotherapy (Hazard Ratio [HR]=1.34, 95%CI=1.23-1.45, P<0.001), initial stage and adjuvant hormonal therapy (HR=1.36, 95%CI=1.22-1.50, P<0.001), and disease-free interval and progesterone receptor positivity (HR=1.02, 95%CI=1.00-104, P=0.043).

3. Effect of Treatment on Overall Survival with Metastatic Disease

When grouped by treatment (Fig 7) the median survival of patients treated with CCT was 25.6 months (95% CI = 23.1-33.2 months), and for patients treated with HDCT/AHPCT was 28.1 months (95% CI = 26.4-36.1 months). This difference was not statistically significant by univariate testing (unadjusted P = 0.39 by logrank).

Fig 7. Overall Survival by Treatment Group



Using the Cox proportional hazards model[61] with forward stepwise regression analysis to adjust for the effects of multiple potential confounding variables, a highly significant treatment effect emerged in favor of HDCT/AHPCT for overall survival (HR=0.62, 95% CI=0.27-0.97, P=0.008). Other variables that had an independent prognostic effect on overall survival are shown in table 10. Disease sites in this model include sites of disease at the time of initiation of chemotherapy.

Variable	P Value (Univariate)	P Value (Multivariate)	HR (Multivariate)	SE	95% CI
Treatment	0.964	0.008	0.62	0.181	0.27-0.97
Estrogen Receptor Status	0.008	0.490	NC	NC	NC
Progesterone Receptor Status	0.002	0.043	0.71	0.165	0.39-1.03
Prior Adjuvant Chemotherapy	< 0.001	0.025	1.25	0.098	1.06-1.44
Prior Adjuvant Hormone Therapy	0.130	0.010	1.69	0.196	1.31-2.07
Disease-Free Interval	0.006	< 0.001	0.99	0.004	0.98-0.99
Metastatic Hormones	0.008	0.651	NC	NC	NC
Number of Metastatic Hormones	< 0.001	< 0.001	0.72	0.088	0.55-0.90
Liver Metastases	< 0.001	0.009	1.65	0.185	1.29-2.01
Lung Metastases	0.145	0.062	1.43	0.188	1.06-1.80
Pleural Metastases	0.020	0.013	2.19	0.288	1.63-2.76
Number of Metastatic Sites	0.004	0.467	NC	NC	NC
Visceral Metastases	0.002	0.669	NC	NC	NC
Status after Chemotherapy	< 0.001	0.001	0.68	0.121	0.45-0.92

HR=hazard ratio; SE=standard error; 95% CI=95% confidence interval;
NC=not calculated by BMDP software

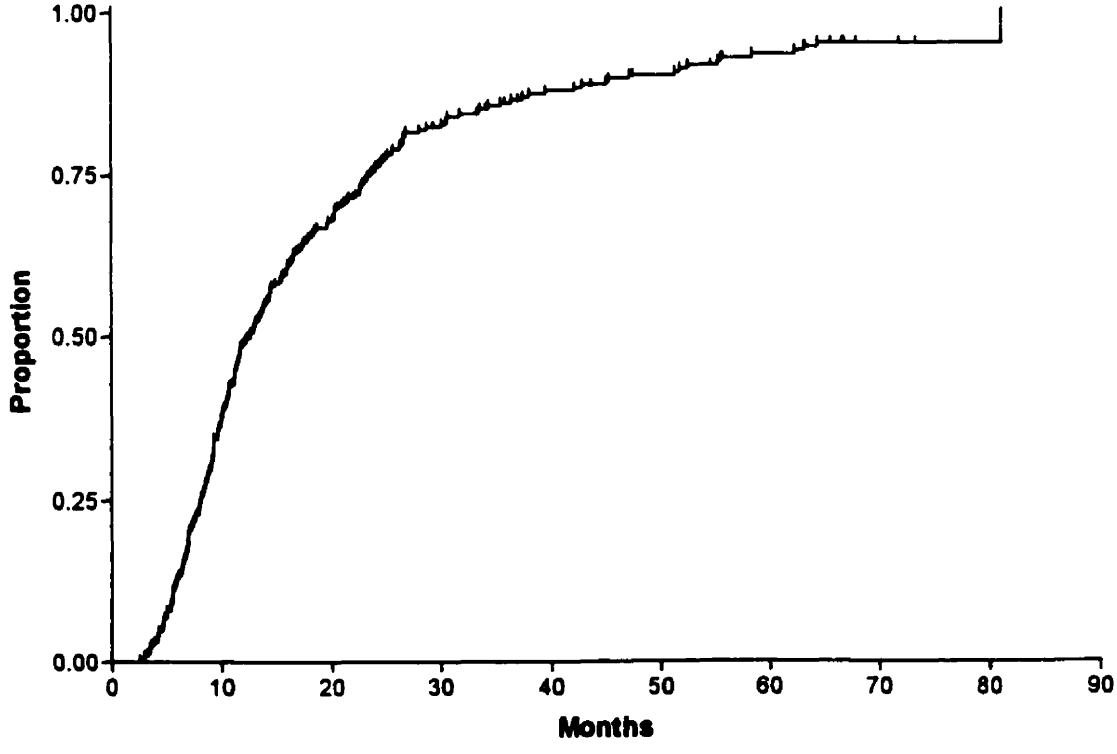
Exploratory analyses for the presence of interaction terms between treatment and other independently significant variables revealed the presence of an interaction between treatment and response to chemotherapy (HR=2.08, 95%CI=1.59-2.57, P=0.003).

v) Time to Failure Outcomes

1. Time to Failure; all Patients

The median duration of time to failure for all patients after chemotherapy (Fig 8) was 12.4 months (95% CI = 11.2-14.3 months).

Fig 8. Time to Failure; All Patients



2. Prognostic Factors for Time to Failure after Initiating Chemotherapy

Using the Cox proportional hazards model[61] and forward stepwise regression analysis to adjust for the effects of multiple potential confounding variables (excluding type of treatment), those variables which had an independent effect on time to failure were determined and are shown in table 11.

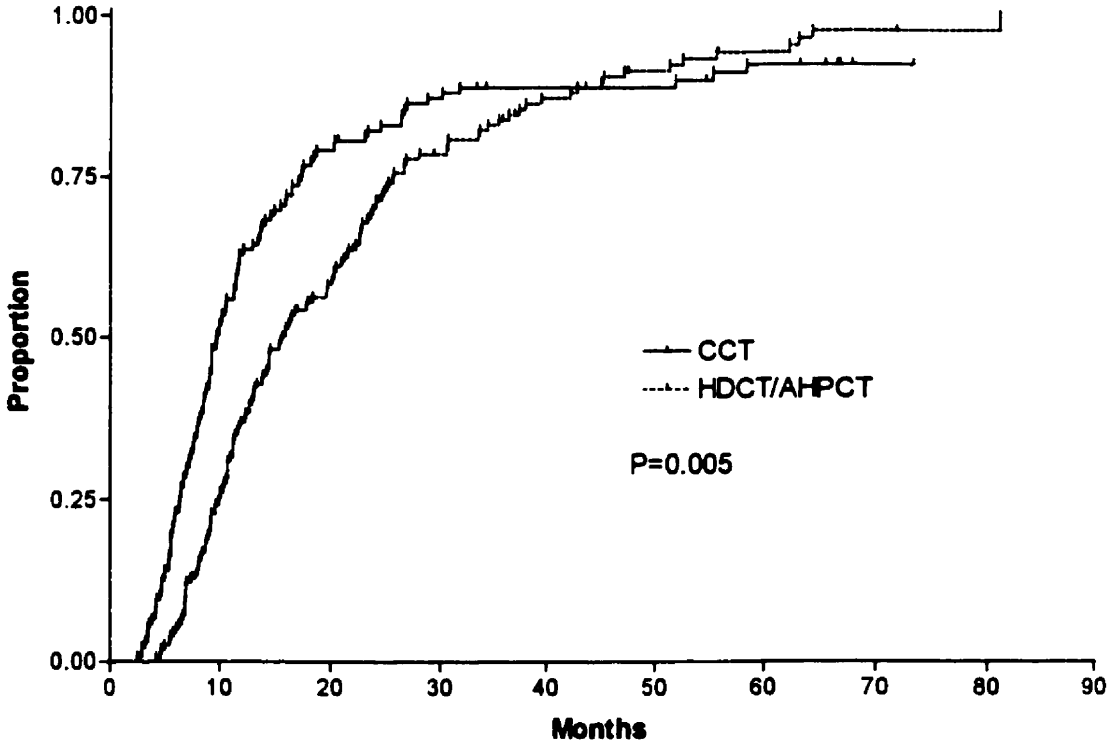
Variable	P Value (Univariate)	P Value (Multivariate)	HR (Multivariate)	SE	95% CI
Progesterone Receptor Status	0.155	0.029	0.73	0.144	0.46-1.01
Prior Adjuvant Chemotherapy	< 0.001	0.003	1.29	0.084	1.12-1.46
Prior Adjuvant Hormone Therapy	0.051	0.166	NC	NC	NC
Number of Sites of Disease	< 0.001	< 0.001	1.28	0.071	1.14-1.42
Liver Metastases	< 0.001	< 0.001	1.62	0.180	1.27-1.97
Lung Metastases	0.002	0.138	NC	NC	NC
Pleural Metastases	0.049	0.705	NC	NC	NC
Locoregional Disease	0.015	0.010	NC	NC	NC
Visceral Disease	<0.001	0.508	NC	NC	NC

HR=hazard ratio; SE=standard error; 95% CI=95% confidence interval;
NC=not calculated by BMDP software

3. Effect of Treatment on Time to Failure

When grouped by type of treatment (Fig 9) the median time to failure for patients treated with standard chemotherapy was 9.8 months (95% CI=8.8-11.4 months), and for patients treated with high-dose chemotherapy was 15.6 months (95% CI=13.3-19.7 months). This difference was statistically significant in univariate testing (unadjusted P=0.005 by logrank).

Fig 9. Time to Failure by Treatment Group



The variables representing "treatment" and "status after chemotherapy" were then added to the analysis, and again the Cox proportional hazards model[61] was used to determine whether or not this univariate effect of treatment remained statistically significant in the multivariate model. This analysis revealed that a highly significant treatment effect in favor of high-dose therapy remained (HR=0.54, 95% CI=0.24-0.84,

P<0.001). Other variables that had an independent prognostic effect on time to failure in this model are shown in table 12.

Variable	P Value (Univariate)	P Value (Multivariate)	HR (Multivariate)	SE	95% CI
Treatment	0.049	< 0.001	0.54	0.166	0.24-0.84
Estrogen Receptor Status	0.186	0.006	0.64	0.166	0.34-0.94
Prior Adjuvant Chemotherapy	< 0.001	0.012	1.24	0.086	1.07-1.41
Prior Adjuvant Hormone Therapy	0.051	0.051	1.44	0.182	1.09-1.79
Number of Sites of Disease	<0.001	0.096	1.14	0.079	0.98-1.30
Liver Metastases	<0.001	0.018	1.56	0.186	1.20-1.92
Lung Metastases	0.002	0.064	1.42	0.186	1.05-1.79
Pleural Metastases	0.049	0.786	NC	NC	NC
Locoregional Disease	0.0152	0.190	NC	NC	NC
Visceral Disease	<0.001	0.300	NC	NC	NC
Status after Chemotherapy	<0.001	<0.001	0.62	0.11	0.41-0.83

HR=hazard ratio; SE=standard error; 95% CI=95% confidence interval;
NC=not calculated by BMDP software

When the variable representing treatment was added, the significance of the variable representing number of sites of disease was reduced considerably (P=0.096). It was recalled at this point that the median number of sites of disease was fewer in the HDCT/AHPCT group. Since treatment allocation was not random, this disparity between study groups (number of disease sites) could have represented the existence of a form of confounding or bias involving the selection of a particular therapy and the number of sites of disease. In other words, physicians may have (consciously or unconsciously) selectively referred patients with fewer sites of disease more often for HDCT/AHPCT, thinking they would be the best candidates for this type of therapy. In an attempt to separate out this possible factor, a series of stratified analyses were run to see if eliminating variability in the potentially confounding variable (number of disease sites) affected the independent significance of the effect of treatment. This analysis could only be performed for a limited set of strata (one and two sites of disease, representing 75% of all patients) because of power/sample size restrictions caused by the distribution of patients in the other strata. Despite this, in both cases elimination of the variability in number of disease sites did not significantly affect the independent significance of treatment (table 13). This suggests that the effect of high-dose therapy is not explained by the existence of selection bias (on the part of physicians) based on the number of sites of disease.

[redacted]

Number of Sites of Disease	N	P Value (HDCT/AHPCT)	HR (HDCT/AHPCT)	SE	95% CI
0-6 (original data)	237	<0.001	0.54	0.166	0.24-0.84
1	104	0.018	0.54	0.253	0.04-1.04
2	74	<0.001	0.40	0.272	0.00-0.93

HR=hazard ratio; SE=standard error; 95% CI = 95% confidence interval; HDCT/AHPCT=high-dose chemotherapy/autologous hematopoietic progenitor cell transplantation

vi) Survival after HDCT/AHPCT

The median duration of survival after HDCT/AHPCT (Fig 10) was 16.5 months (95% CI=13.7-21.7 months). The median overall survival after transplantation for patients treated in Omaha was 15.3 months (95% CI=12.2-23.5 months) and for patients treated in Sudbury was 16.3 months (95% CI=13.8-25.6 months). There was no statistically significant difference in duration of survival after transplantation when analyzed by centre (Fig 11, P=0.70 by logrank test). The effect of centre remained statistically insignificant in multivariate analysis (P=0.65). The independent effect of various disease related variables on outcome after transplantation are shown in table 14.

Fig 10. Survival after HDCT/AHPCT; All Patients

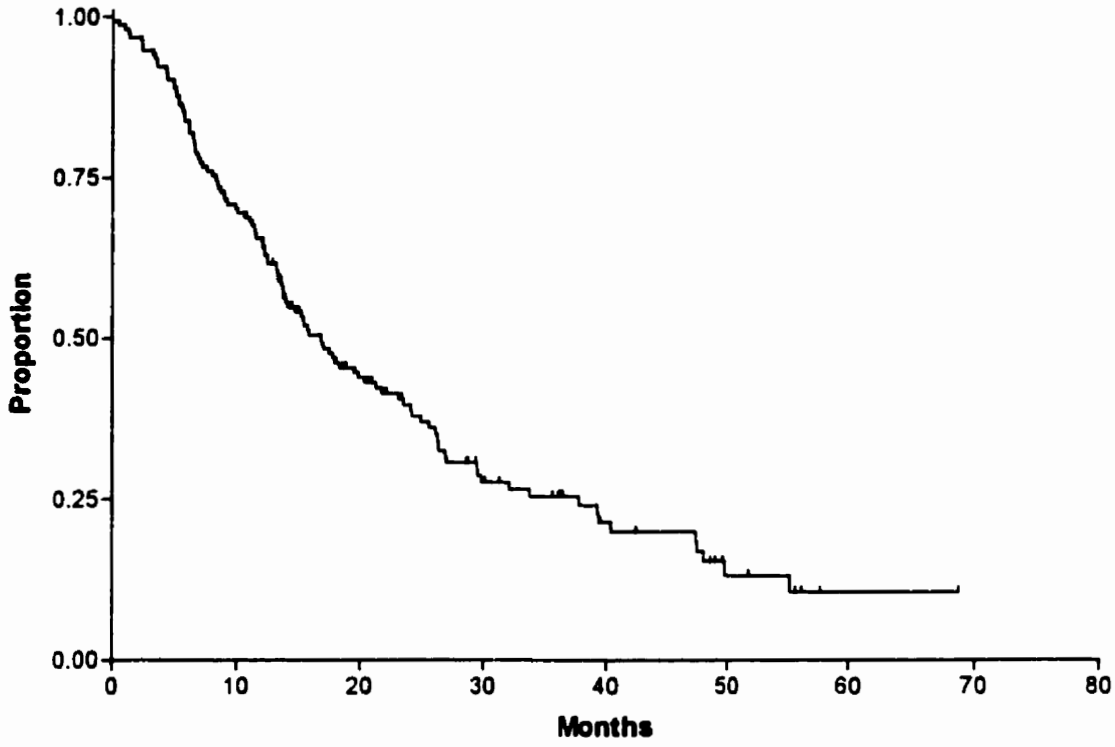
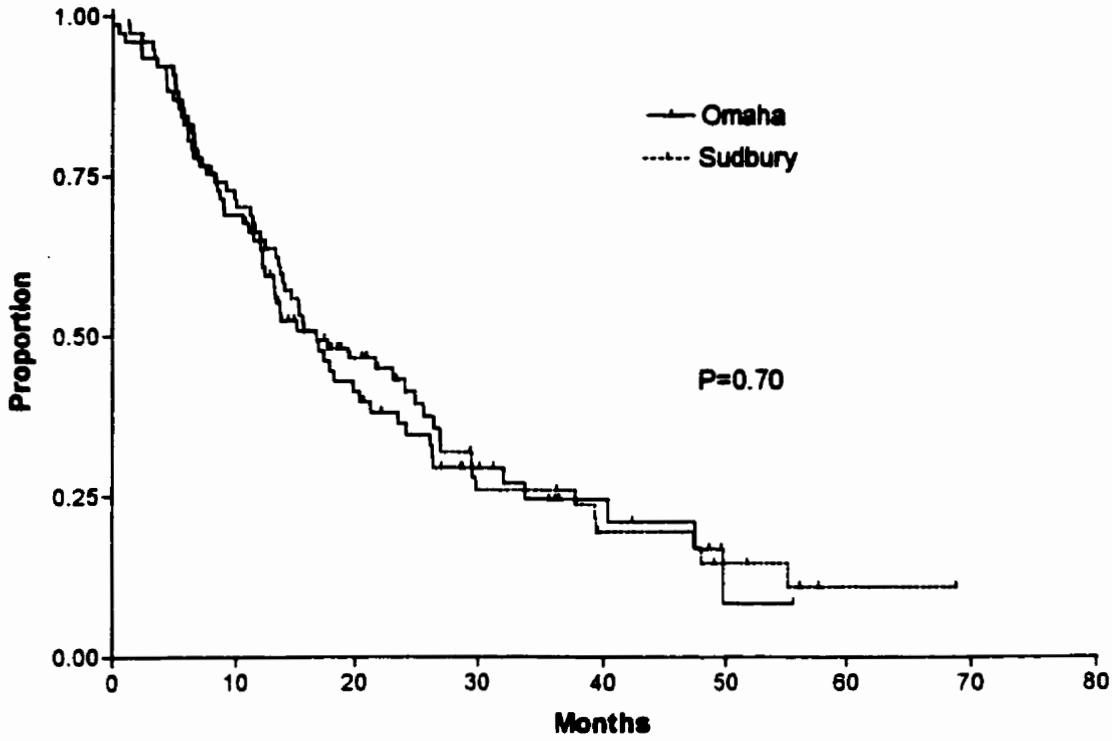


Fig 11. Survival after HDCT/AHPCT by Centre



Variable	P Value (Univariate)	P Value (Multivariate)	HR (Multivariate)	SE	95% CI
Disease-Free Interval	0.162	0.288	NC	NC	NC
Age at Transplantation	0.017	0.008	0.96	0.017	0.93-0.99
Estrogen Receptor Positivity	0.192	0.026	0.54	0.280	0.00-1.08
Prior Adjuvant Chemotherapy	0.042	0.349	NC	NC	NC
Liver Metastases	0.001	< 0.001	3.13	0.290	2.54-3.86
Bone Metastases	0.522	0.019	1.78	0.246	1.30-2.26
Visceral Metastases	0.026	0.432	NC	NC	NC

HR=hazard ratio; SE=standard error; 95% CI=95% confidence interval;
NC=not calculated by BMDP software

vii) Prognostic Factors For Survival after HDCT/AHPCT (including transplant related variables)

The multivariate model was re-run including the effect of centre, conditioning (high-dose chemotherapy) regimen, the presence/absence of bone marrow involvement at the time of HDCT/AHPCT, and infused progenitor cell product (bone marrow versus peripheral blood versus both) (table 15). None of these additional transplant related variables had a significant influence on survival post transplantation.

Variable	P Value (Univariate)	P Value (Multivariate)	HR (Multivariate)	SE	95% CI
Age at Transplantation	0.017	0.008	0.96	0.02	0.93-0.99
Estrogen Receptor Positivity	0.192	0.026	0.54	0.28	0.00-1.08
Prior Adjuvant Chemotherapy	0.042	0.349	NC	NC	NC
Number Metastatic Hormones	0.041	0.178	NC	NC	NC
Liver Metastases	0.001	< 0.001	3.13	0.29	2.56-3.70
Bone Metastases	0.522	0.019	1.78	0.24	1.31-2.25
Visceral Metastases	0.026	0.432	NC	NC	NC
Centre	0.413	0.645	NC	NC	NC
Conditioning Regimen	0.222	0.378	NC	NC	NC
Marrow at Transplantation	0.206	0.815	NC	NC	NC
Rescue Product	0.175	0.807	NC	NC	NC

HR=hazard ratio; SE=standard error; 95% CI=95% confidence interval;
NC=not calculated by BMDP software

VII. Discussion

This retrospective cohort study has attempted to measure the effect of HDCT/AHPCT as compared with CCT on the overall survival and time to failure for patients with metastatic breast cancer. Recognizing limitations of this study design, all possible attempts were made to minimize possible sources of selection bias, and between-group differences in all known confounding/prognostic factors were adjusted for in the analyses using multivariate techniques. In one instance (the effect of treatment on time to failure), the use of a stratified analysis was employed to rule out the possibility of an effect of selection bias on a confounding variable (number of sites of disease). Calculations of median duration of survival, as well as determinations of independent prognostic factors for both recurrence of disease and for survival after recurrence of breast cancer are in general agreement with the published literature, lending some degree of credibility/validity to the overall content of the database. From an experimental standpoint, the results of this study suggest that the use of HDCT/AHPCT is associated with a beneficial effect on both overall survival and time to failure as compared to CCT, and that this effect is independent of known prognostic factors, as is discussed below.

i) Prognostic Factors for Time to Recurrence (Disease-Free Interval)

Although age at diagnosis, number of positive axillary lymph nodes, initial clinical stage, and both estrogen and progesterone receptor status were all statistically significant in univariate analysis, only advancing initial clinical stage (conferring an increased risk; HR=1.58) and progesterone receptor positivity (conferring a protective effect; HR=0.77) retained statistical significance in multivariate analysis (table 6). Advancing age was of

borderline statistical significance (HR=1.02, P=0.055). The elimination of number of positive axillary lymph nodes as a variable with independent significance reflects the fact that axillary lymph node status is incorporated into clinical staging. These findings are in general agreement with previously reported literature,[19,26,27,30-32] however previous prognostic studies have concentrated more on the influence of estrogen receptor status, often ignoring the effect of progesterone receptor status. In this study, receptor status was an independent prognostic factor for outcome in every analysis but one. In several of the analyses however, progesterone receptor status appeared to override or replace the significance of estrogen receptor status. A careful review of the distribution of receptor status reveals that estrogen and progesterone receptor status were concordant (i.e., both positive in 124 patients and both negative in 71 patients) for a total of 195/255 patients (76%, Chi square = 66.26, D.F.=1, P<0.001). When the same multivariate analyses were re-run eliminating the variable representing progesterone receptor status, estrogen receptor status entered into the model with similar hazard ratios and significance levels. In attempting to run the analyses with an interaction term for receptor status the analysis was aborted by the software algorithm because of co-linearity between terms. All of these observations suggest the presence of a biologically expected high level of concordance between estrogen and progesterone receptor status.

Although estrogen receptors have traditionally received more attention in the area of prognostication/response to endocrine therapy, some investigators feel that progesterone receptors may actually be a better predictor of the same.[138,139] The exposure of breast cancer cells to estrogen leads to an increase in the production of progesterone receptors within those cells,[138] suggesting that progesterone receptor bearing cells contain estrogen receptors that are not just structurally/physically present, but rather are also biologically functional. Thus progesterone receptor positivity may be

a marker for estrogen receptor functionality, and for that reason may be more predictive of endocrine responsiveness and prognosis.[139] The importance of progesterone receptor status independent of estrogen receptor status has been clinically demonstrated in both retrospective[140,141] and prospective[142] studies.

ii) Overall Survival Outcomes

1. Overall Survival/Predictors of Overall Survival

The median overall survival duration (Fig 6) of 27.4 months is within the range of that reported for survival in patients with metastatic breast cancer.[48-50,63,143,144] Independent prognostic factors associated with longer survival at the point of initial metastatic disease (table 9) included progesterone receptor positivity (HR=0.62), longer disease-free interval (HR=0.98), the presence of bone metastases (HR=0.59), and the existence of locoregional disease (HR=0.45). Variables independently associated with a shorter survival included prior adjuvant chemotherapy (HR=2.34), prior adjuvant hormone therapy (HR=2.33), the presence of liver metastases (HR=1.68), and increasing number of disease sites (HR=2.06). Again, these findings are consistent with previously reported literature.[48,50,143,144] Interaction or effect modification was found between initial stage and adjuvant chemotherapy (HR=1.34) and initial stage and adjuvant hormonal therapy (HR=1.36). These interactions suggest that the magnitude of effect of prior adjuvant therapy on survival was differentially affected by stage at diagnosis. The presence of an interaction between disease-free interval and progesterone receptor positivity (HR=1.02) implies that the magnitude of effect of disease-free interval on survival was differentially affected by progesterone receptor status.

The adverse effect of prior adjuvant hormone therapy may at first glance appear counter-intuitive, since the use of adjuvant hormone therapy should imply the treatment of receptor positive patients; generally a group with a better overall prognosis. However, two factors may explain this result. First, patients who develop recurrent disease despite adjuvant therapy likely have disease that is in a biologic sense more aggressive than simple measures of prognosis (such as receptor status) may have implied. Failure of disease in the presence of (or after) an adjuvant therapy suggests acquired resistance and such patients are often refractory to further therapies. Second, a close examination of the baseline characteristics of all patients reveals that of 163 estrogen receptor positive patients in the study, only 40 received adjuvant hormone therapy (i.e., that 123 receptor positive patients did not receive adjuvant hormone therapy). An analysis for the presence of an interaction between estrogen receptor status and having received adjuvant hormone therapy produced a negative interaction (HR=0.28). This suggests that survival with metastatic disease was better in receptor positive patients who had not received prior adjuvant hormone therapy, which is consistent with the hypothesis of acquired resistance to therapy.

A final observation from this analysis is that although visceral disease has been consistently reported as an independent negative prognostic factor in this setting, visceral disease was not seen to be an independent predictor of poor outcome in this multivariate model. The variable representing visceral disease was a composite of several sites of visceral disease (lung, liver, bowel etc.), and was represented by liver metastases in 36/54 patients (67%). In the stepwise regression model, the elimination of the variable representing liver metastases caused the variable representing visceral disease to enter the model (HR=1.68, 95% CI= 1.21-2.08, P=0.047). An attempt to identify the presence of an interaction between these two variables resulted in an aborted analysis because of a high degree of concordance between the two variables.

These results suggest that the inability to separate out the independent effects of visceral disease and liver metastases results from a high level of concordance between these two variables, as expected by the composite nature of the variable visceral disease, and the high level effect of the presence of liver metastases.

2. Effect of Treatment on Overall Survival with Metastatic Disease

Overall survival was defined as the point in time at which disease recurrence was identified until death from any cause to allow treatment related deaths (more likely to occur in the HDCT/AHPCT group) to be captured as outcomes. Defining survival in this way makes the endpoint less prone to any form of temporal bias (e.g., if one group had begun chemotherapy on average later than the other group). Although the unadjusted life table analysis for survival revealed no significant difference between treatments (Fig 7; P=0.39 by logrank), the addition of the treatment variable to the multivariate overall survival model produced a statistically significant effect in favor of HDCT/AHPCT (table 10; HR=0.62, 95% CI=0.27-0.97, P=0.008). The hazard ratio of 0.62 implies that patients treated with CCT were likely to die approximately 1.6 fold faster than those receiving HDCT/AHPCT. This difference in probability of survival would likely be considered clinically relevant by most patients (unpublished observations).

The explanation for the large difference between univariate and multivariate analyses appears to lie in the between-group distribution and effect of other variables with independent prognostic significance for survival. Except for bone metastases (equally distributed between both groups), variables that conveyed a protective effect on survival (progesterone receptor positivity, longer disease-free interval, and locoregional disease) were seen in a smaller proportion of patients in the HDCT/AHPCT group. Similarly, variables which conveyed the most quantitatively significant degree of

adverse effect on survival (prior adjuvant chemotherapy, HR=2.34, and prior adjuvant hormone therapy, HR=2.33) were seen in a larger proportion of patients in the HDCT/AHPCT group. Although the CCT group contained more patients with liver metastases (HR=1.68) and patients with a greater number of disease sites (HR=2.06), the overall combined prognostic weight of these variables suggests that the HDCT/AHPCT patients were a worse prognostic group with respect to overall survival, accounting for the large difference between univariate and multivariate analyses.

Other treatment related variables in this model which were of prognostic importance included number of prior metastatic hormonal therapies (which conveyed a protective effect; HR 0.72), and status after chemotherapy (HR 0.68) which also conveyed a protective effect for patients with more complete degrees of response. The beneficial effect of number of prior metastatic hormones likely represents the identification of patients with hormone sensitive disease; a group with generally more indolent disease and a better overall prognosis.[145] Similarly, the beneficial effect of a better response to chemotherapy implies a greater degree of disease sensitivity; also a group with a better overall prognosis. This independent beneficial effect of chemotherapeutic response is consistent with data reported in the breast cancer literature in both transplant[129,130,132] and non-transplant[134] settings, and is in agreement with the commonly observed principle in oncology that "responders" have better outcomes compared to "non-responders".[60]

Finally, the presence of a positive interaction between treatment and response to chemotherapy implies that patients who had better responses after three to four cycles of chemotherapy had better outcomes with HDCT/AHPCT than with CCT. This could be a manifestation of a dose-response effect.

iii) Time to Failure Outcomes

Since failure of disease control is a less objective outcome and more prone to measurement bias, less emphasis can be placed on the results of this portion of the analysis. However, prolongation of disease control and prolongation of survival are (where appropriate statistical power exists) in general positively correlated in most malignant diseases. It is important to emphasize the differences in the calculation of time to failure and overall survival in this particular study. The effect of treatment on overall survival looked at the effect of treatment on the entire survival duration of a patient (from initial recurrence to death). The effect of treatment on time to failure denotes only a portion of time during the life of a patient; namely from the point of initiation of chemotherapy until the first signs of progression of disease thereafter. Because of the increased possibility of therapy related death from HDCT/AHPCT, death was included as an event in this outcome measure.

1. Time to Failure/Predictors of Time to Failure

The median duration of time to failure for all patients (Fig 8) was 12.4 months, which is within the range of that reported in the literature.[1] Variables which had independent significance for time to failure at the initiation of chemotherapy (table 11) included progesterone receptor status (HR 0.73), prior adjuvant chemotherapy (HR 1.29), number of sites of disease (HR 1.28), and the presence of liver metastases (HR = 1.62). The negative effect of prior adjuvant chemotherapy is likely related to the acquisition and clinical expression of disease resistance as has been discussed previously. The negative effect of increasing number of disease sites likely reflects the adverse prognosis associated with increasing tumor burden, and the negative effect of

liver metastases reflects on the presence of the illness in a critical organ, both generally associated with shorter a time to progression independent of therapy.[129,132]

2. Effect of Treatment on Time to Failure

When grouped by treatment in univariate analysis (Fig 9), the median duration of time to failure was 9.8 months for patients undergoing CCT, and 15.6 months for patients undergoing HDCT/AHPCT (P=0.005 by logrank test). Adding treatment related variables to the multivariate model (table 12) revealed a continued and independent beneficial effect for HDCT/AHPCT on time to failure (HR 0.54). The hazard ratio of 0.54 implies that patients treated with CCT were approximately 1.8 fold more likely to experience progression after beginning chemotherapy. This difference would also likely be considered clinically relevant by most patients when measured against the toxicity of the treatment. Other variables with independent significance for failure in this model included prior adjuvant chemotherapy (HR 1.24), prior adjuvant hormone therapy (HR 1.44), the presence of liver metastases (HR 1.56), status after chemotherapy (HR 0.62), and estrogen receptor positivity (HR 0.64). When it was seen that the addition of the treatment variable to the model substantially reduced the independent significance of the variable representing number of sites of disease, the between-group distribution of number of sites of disease was reviewed. It was noted that the median number of disease sites was significantly higher in the CCT group, and this was felt likely to represent the existence of a high degree of confounding between type of treatment and number of sites of disease. This high degree of confounding, if present, would reduce to some degree the ability of the analysis to truly identify and quantify any effect of treatment independent of the number of sites of disease, and could reflect a form of selection bias (i.e., that patients selected for HDCT/AHPCT had a lesser amount of disease in general). An attempt was made to separate out the possible effect of

selection bias by performing a stratified analysis on number of disease sites. This was done to eliminate the between-group variability in the number of disease sites while retaining appropriate statistical power for the analysis to measure the effect of treatment (sample size and between-group distribution limitations allowed for this analysis to be performed only on patients with one or two sites of disease). These results suggested that the effect of treatment was independent of the between-group variability in number of disease sites, regardless of the level of stratification (table 13), and supports the conclusion that the positive result associated with HDCT/AHPCT was a true independent therapeutic effect.

It was also noted that with respect to time to failure the life-table analysis curves remain separated in favor of the HDCT/AHPCT group until approximately 40 months, at which point the curves crossed-over and there appeared to be a progression-free benefit for patients in the CCT group. At this late point in the curves, the subjects consist of 11 patients in the standard chemotherapy group, and 15 in the high-dose chemotherapy group. An exploratory univariate sub-analysis was performed on this group of patients using only those variables shown to have independent significance for progression in the prior multivariate analysis. This analysis revealed no significant differences between the groups with respect to four of five independent prognostic factors (estrogen receptor status, prior adjuvant chemotherapy, prior adjuvant hormone therapy, presence of liver metastases), but revealed a significant difference between groups with respect to status after chemotherapy. It was discovered that at this point in the curves nine of 11 patients in the CCT group were either NED or in CR after chemotherapy, whereas only one of the 15 patients in the HDCT/AHPCT group was in CR after chemotherapy (P=0.001). This effect would be considered significant even after adjusting for the effect of multiple testing (Bonferroni correction would accept a P value of 0.01 or less as being significant based on the performance of 5 statistical tests

in this sub-group of patients). The confounding effect of response to chemotherapy in this small group of patients on the distant portion of the curve likely explains the crossover in the curve in favor of the CCT group, as more patients in the CCT group at this point had a factor (response to treatment) which was predictive of a better outcome independent of the type of therapy (HDCT/AHPCT vs. CCT).

iv) Survival after HDCT/AHPCT

Variables which had independent predictive effects on survival after HDCT/AHPCT (table 14) included age at transplantation (HR 0.96), estrogen receptor positivity (HR 0.54), the presence of liver metastases (HR 3.13), and the presence of bone metastases, which surprisingly were associated with a poor outcome (HR 1.78) for reasons unclear. The median overall survival of all HDCT/AHPCT patients after initiation of chemotherapy was 16.5 months (Fig 10), and was not significantly different for patients treated in Omaha, who had a median survival of 15.3 months, as compared with patients treated in Sudbury, who had a median survival of 16.3 months (Fig 11; P=0.70 by logrank test). Acknowledging limitations on statistical power as a result of the sample size, the effect of centre (table 15) remained statistically insignificant in the multivariate model after the addition of a number of treatment related variables which included the high-dose chemotherapy regimen (conditioning regimen) used, the presence of metastatic disease in bone marrow at transplantation, and the rescue product used (bone marrow vs. peripheral blood). Again, acknowledging power/sample size limitations, the response to chemotherapy pre-transplant was not indicative of a better outcome post transplant, and the time from disease recurrence to first chemotherapy (a form of potential temporal bias indirectly reflecting disease biology) also had no measurable effect on survival after transplantation in this group.

In summary, the results of this retrospective cohort analysis suggest that a measurable effect for HDCT/AHPCT for patients with metastatic breast cancer does exist, specifically in the form of progression-free and overall survival advantages when compared with CCT. With few exceptions, the datasets seem to agree with previously published literature with respect to all major endpoints analyzed, lending a reasonable degree of credibility to the datasets themselves. It is still possible that subtle differences in selection and confounding variables not measurable by the study design account for some measure of these results, but any such effect is not quantifiable given the constraints of this study. Notwithstanding issues relating to bias and confounding (discussed below), the study has produced generally consistent representations of biologic understanding and the literature, and lends some further credibility to the effect of HDCT/AHPCT in metastatic breast cancer.

v) The Effect of Bias

1. Selection Bias

The potential effect of selection bias was perhaps the most difficult and important aspect of this study design, given that the eligibility of patients for the control group had to be determined retrospectively, occasionally using less sensitive tools. In considering this, every possible attempt was made to eliminate patients from the control group who would not have been candidates for HDCT/AHPCT. The criteria developed were matched as carefully as possible to the criteria used for the experimental group, and were considered to be the best available retrospective measures of disease, treatment response, and performance status. Having a second individual review charts for concordance was considered, but given the expertise and extent of time required to properly review charts this was not feasible. Although in some cases surrogate

measures of eligibility had to be used (e.g., chest radiographs and clinical histories to rule out significant cardiac disease where no MUGA scan was present), application of the predetermined control group eligibility criteria retrospectively to a randomly selected group of potential control patients resulted in the elimination of 79 of 214 patients or nearly 40% of all potential control patients reviewed, suggesting a reasonable degree of stringency in the selection of controls. The most common reasons for ineligibility of controls (non-response to chemotherapy, 31 patients; known CNS disease, 16 patients; and inadequate follow-up, 9 patients) together accounted for 56 of 79 patients, or 70% of those deemed ineligible. The most common reasons for ineligibility in the experimental groups were CNS metastases, and non-response to chemotherapy, which were fairly easily evaluable in controls. The number of patients excluded from both the experimental and control groups because of poor organ function or performance status (areas where the control group eligibility criteria were weakest) were few, and as such did not likely represent a major source of selection bias. Temporal biases were minimized by choosing control patients treated over the same calendar time period (1991-1995), such that the availability and use of other adjunctive therapies or newer chemotherapeutic agents (which could have had an independent effect on outcome) would likely have been equally distributed between the two groups. Despite these attempts to minimize selection bias, different methods and temporal sequences were used to select experimental and control groups, and this remains perhaps the most important unquantifiable source of potential error or difference between the two study groups. A probable effect of selection bias was seen in the observation of the high degree of correlation between treatment and number of sites of disease for time to failure, as was discussed in an earlier section. However this particular selection factor did not appear to account for differences in outcome between the two groups.

2. Measurement Bias

The other major potential source of bias in a study such as this is measurement bias. The majority of baseline or therapy related prognostic variables were fairly objective in their determinations/measurements (e.g., initial stage, prior adjuvant therapies etc.), and were not likely to be subject to any substantial degree of measurement bias. A few such variables did have inherent weaknesses in their measurement/assignment (e.g., what duration of therapy/disease control constituted a "trial" of a particular hormone therapy) and could have had some unquantifiable effect if systematically measured differently between centres or groups. The variable most likely to have been subject to some form of measurement bias would have been the variable representing response to treatment since responses were not always rigorously measured, particularly in the control group, and as such may have been overestimated to some degree in that group. Also, determinations of progression/recurrence of disease after treatment could theoretically have been biased by differences in follow-up and imaging practices; however the observation of a relatively small difference in the hazard ratios for the effect of treatment on recurrence (the softer endpoint, HR=0.54) as compared with survival (the harder endpoint, HR=0.62) suggests that this was not likely an important source of bias. This is especially true when one considers that the effect of treatment on overall survival was measured over the entire period of metastatic disease, whereas the effect of treatment on failure was measured over a smaller time period (between first chemotherapy and first failure).

vi) Adjusting for the Effect of Confounding Variables

Patients in this study were of course not randomly allocated to treatment groups. Rather, they were selected and collected from three separate databases with differing demographics and geographic locations. Therefore it was expected that measurable differences would exist between patients. Data on all known major confounding variables was collected and recorded. Notwithstanding previous considerations of the possible sources of measurement bias, these data could be used both to establish the validity/credibility of the databases (i.e., to compare independent prognostic variables with previously reported literature), and to adjust for differences between groups in trying to measure any effect of high-dose therapy. The combined database appeared to contain all appropriate major prognostic variables, and with very few exceptions (e.g., the adverse effect of the presence of bone metastases on survival after HDCT/AHPCT) their importance in these analyses were in agreement with that reported in the literature.

Most significant with respect to the presence of confounding variables is the possibility that certain unknown or unmeasured confounding variables could have been differentially distributed between study groups. If clinically important, such variables could have accounted in some measure for the observed outcomes of the study. Such unmeasured confounding variables could have been biologic characteristics or treatment related characteristics:

1. Biologic Characteristics

Certain prognostic biologic characteristics of both groups are not known. For example, while it has long been known that the human epidermal growth factor receptor-2 (HER-2/neu) is overexpressed in approximately 30% of women with node positive breast cancer,[146] it has only been recently that its clinical prognostic and therapeutic significance has begun to be understood. Although overexpression of this receptor is associated with a poor prognosis and shorter time to relapse,[147] overexpression may also be a predictor of chemotherapeutic dose-responsiveness[148] and could have had an unmeasured effect if differentially distributed between groups.

2. Treatment Related Characteristics

Virtually all patients would have received other types of chemotherapy after failure of first-line treatment, and it is possible that these treatments were differentially distributed between study groups despite equivalent temporal selection of the study groups. This data was known for patients in the Ottawa and Sudbury groups, but had not been recorded for patients from Omaha. Also, it was not certain that this component of the Sudbury database was complete, and therefore this information could not be used with confidence. Of particular importance in this regard is the existence of some newer chemotherapeutic agents (e.g., paclitaxel, docetaxel, vinorelbine). The use of these newer agents may actually confer small survival advantages to patients who receive them as was suggested by a recent randomized trial of docetaxel in metastatic breast cancer.[149] Such treatments could have been differentially received by one of the groups subsequent to the first chemotherapy failure. If such treatments had the ability

to prolong survival, their use could have contributed an unmeasured effect to the overall survival outcome.

Unfortunately, even if this data were available and complete, this particular study design would not have been able to reliably measure such an outcome as an effect of treatment. Since treatments received subsequent to first chemotherapy failure were both time dependent and not randomly allocated, an alternate explanation for any apparent survival benefit from those treatments could equally be the effect of selection bias (i.e., that those patients with better prognosis disease survived long enough to receive such agents).

vii) Methodologic Comparisons to the Bezwoda Trial

Though not randomized, this study has dealt with some of the imperfections considered to exist in the randomized Bezwoda trial.[135] First, the CCT arm in the Bezwoda study consisted of a regimen which is not be considered "standard" by North American investigators, and in this study demonstrated response rates which were inferior to those expected with more usual modern conventional regimens. This choice of regimen therefore may have potentially biased the results toward superiority for the high-dose arm. The regimens used in the control group for the present study were all recognized conventional chemotherapy regimens with known published response rates. Second, in the Bezwoda study patients who achieved complete responses to chemotherapy were begun on hormonal therapy with tamoxifen. This may have biased outcomes toward the experimental group, since there were significantly more complete responses in that group. The present study recorded (and adjusted for) the use of and number of hormones, and therefore less likely contributed to error in measurement of the outcomes. Third, certain independent prognostic variables for outcome in

metastatic breast cancer (e.g., disease-free interval) were not measured or presented in the Bezwoda study, and the number of disease sites (an independent prognostic variable for outcome) was presented as means rather than medians, which may have been misleading given that this type of data in a small sample is unlikely to have been normally distributed. These were not issues in the present study. Fourth, the results of statistical tests for time to progression and overall survival were not expressed with any associated P values, and despite some baseline differences between groups with respect to important prognostic variables (e.g., estrogen receptor status, presence of bone metastases), no multivariate analysis was performed to adjust the outcome for these differences. Again, these were not issues in the present study. Finally, the duration of follow-up in the Bezwoda trial was not adequate to evaluate any potential plateau on the survival curve. None of the outcome curves in the present study display any meaningful plateaus. Therefore, notwithstanding published data from the Bezwoda trial or data from the present study, the superiority of high-dose therapy must still be considered unproven. One final point worth mentioning is that the Bezwoda trial utilized two cycles of moderately high-dose therapy, taking advantage of the concept of log-kill. This concept has not been compared head to head in any high-dose chemotherapy trials (randomized phase II or phase III) for metastatic breast cancer, and could conceivably have contributed in some positive way to the results of that trial, since this is the biologic premise upon which all potentially curative chemotherapy regimens are based.

viii) Summary and Conclusions

In summary, despite many years of experience and follow-up in high-level academic transplant centres worldwide, controversy remains regarding what benefit, if any, is imparted to patients with metastatic breast cancer who undergo HDCT/AHPCT. The existence of this controversy results principally from a lack of well designed completed randomized controlled trials from which meaningful clinically relevant conclusions can be made.

In attempting to quantify the benefits associated with any new therapy, it is useful to consider the treatment from the point of view of three main therapeutic endpoints of importance in any major illness: the ability to cure, the ability to prolong survival, and the ability to improve the quality of life of patients with the disease. Proponents of HDCT/AHPCT maintain that since no form of therapy for metastatic breast cancer is curative nor convincingly able to prolong life, the most realistic endpoint for patients is to attempt to improve quality of life by decreasing symptoms associated with the disease. CCT given in a cyclic fashion every three to four weeks can achieve this for many patients, and is generally associated with only modest toxicity and a very low risk of treatment related mortality. However, patients often require prolonged periods of treatment (e.g., 12-18 months of therapy) in order to maintain control of their disease. HDCT/AHPCT is associated with initially greater levels of toxicity and risks of treatment related mortality, but can allow patients to achieve a period of time off of treatment (i.e., to achieve an unmaintained remission). This can be a clinically meaningful benefit for certain patients who find repeated cyclic chemotherapy a less desirable way of living with their disease (unpublished observations). Given that HDCT/AHPCT is associated with higher risks, not every eligible patient elects to undergo this type of therapy given

the choice; however, transplant related mortality is now reported by most experienced transplant centres as being approximately five percent.[136]

Opponents of HDCT/AHPCT see this form of treatment as both expensive and toxic, and argue that all benefits purported in uncontrolled trials are merely the result of selection bias on the part of the investigators.[134] Studies attempting to measure economic and quality of life endpoints have been few. A recent attempt to quantify differences in costs between CCT and outpatient-based HDCT/AHPCT from a Canadian perspective demonstrated that HDCT/AHPCT costs approximately $13,000 more in the first year of treatment/follow-up.[150] This difference would likely be considered small by most health economists, and can quantitatively be compared to the now established practice of using monthly pamidronate as an adjunctive therapy for patients with breast cancer and symptomatic bone metastases.

Should ongoing randomized trials in this area eventually conclude that no survival advantage exists for patients who undergo HDCT/AHPCT, this form of treatment might best be considered yet another form of palliative therapy for an incurable illness, and that it should remain an option for patients who prefer to accept the tradeoff of an increased risk of treatment related morbidity/mortality in return for achieving a period of time off of cyclic chemotherapy. Further study regarding the economic and quality of life differences between the two treatments should also help define its role as a therapy for patients with metastatic breast cancer.

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Appendix 1. American Joint Centers for Cancer Staging of Breast Cancer 1992

TNM Definitions

Primary Tumor (T)

Tx: Primary tumor cannot be assessed
T0: No evidence of primary tumor
Tis: Carcinoma in situ
T1: Tumor 2 cm or less in greatest dimension
T1a: 0.5 cm or smaller
T1b: More than 0.5 cm but not more than 1.0 cm in greatest dimension
T1c: More than 1.0 cm but not more than 2.0 cm in greatest dimension
T2: Tumor more than 2.0 cm but not more than 5.0 cm in greatest dimension
T3: Tumor more than 5.0 cm in greatest dimension
T4:Tumor of any size with direct extension to chest wall or skin
T4a: Extension to chest wall
T4b: Edema, ulceration of skin, or ipsilateral satellite skin nodules
T4c: Both T4a and T4b
T4d: Inflammatory carcinoma

Regional Lymph Node Involvement (N)
Clinical

Nx: Regional lymph nodes cannot be assessed
N0: No regional lymph node metastases
N1: Metastases to moveable ipsilateral axillary node(s)
N2: Metastases to ipsilateral axillary node(s) fixed to one another or other structures
N3: Metastases to ipsilateral internal mammary lymph node(s)

Pathologic (pN)

PNx: Regional lymph nodes cannot be assessed
PN0: No regional lymph node metastases
pN1: Metastases to moveable ipsilateral axillary node(s)
pN1a: Only micrometastases (none larger than 0.2cm)
pN1b: Metastases to lymph nodes, any larger than 0.2 cm
pN2: Metastases to ipsilateral axillary node(s) fixed to one another or other structures
pN3: Metastases to ipsilateral internal mammary lymph node(s)

Distant Metastases (M)

Mx: Presence of distant metastases cannot be assessed
M0: No distant metastases
M1: Distant metastases

Stage Grouping

Stage 0: Tis, N0, M0

Stage I: T1, N0, M0

Stage IIA: T0, N1, M0
T1, N1, M0
T2, N0, M0

Stage IIB: T2, N1, M0
T3, N0, M0

Stage IIIA: T0, N2, M0
T1, N2, M0
T2, N2, M0
T3, N2, M0

Stage IIIB: T4, any N, M0
Any T, N3, M0

Stage IV: Any T, any N, M1

Appendix 2. Standard Definitions of Performance Status

(Eastern Cooperative Oncology Group)

0 - Asymptomatic, able to carry out normal activity

1 - Symptomatic with minimal activity, fully ambulatory

2 - Symptomatic, requiring bedrest < 50% of waking hours

3 - Symptomatic, requiring bedrest > 50% of waking hours

4 - Bedridden

Appendix 3. Standard Definitions of Tumor Associated Response Rates

Complete Response (CR):

Defined as the disappearance of all known measurable clinical and radiographic evidence of disease for a minimum of 4 weeks.

Partial Response (PR):

Defined as a greater than 50% decrease in the sum of the products of measured lesions (measured at maximal perpendicular diameters) in the absence of any increase in lesion size and no appearance of new lesions for at least 4 weeks.

Stable Disease (SD):

Defined as a less than 50% decrease in the sum of the products of measured lesions (measured at maximal perpendicular diameters) in the absence of any increase in lesion size and no appearance of new lesions for at least 4 weeks.

Progressive Disease (PD):

Defined as the unequivocal increase by at least 25% in the sum of the products of measured lesions or the appearance of new lesions.

Appendix 4. University of Nebraska Medical Center Eligibility Criteria for High-Dose Chemotherapy/Autologous Hematopoietic Stem Cell Transplantation

Biopsy proven diagnosis of breast cancer, evidence of recurrent/metastatic disease

Female, aged 19-60 years

No evidence of organ dysfunction unrelated to malignancy that would make patients unlikely to tolerate high-dose therapy:

Absolute neutrophil count ≥ 1500; platelet count ≥ 100,000

Adequate renal function (serum creatinine ≤ 1.5 mg/dl and/or creatinine clearance ≥ 60 ml/min

Adequate hepatic function (bilirubin ≤ 2 mg/dl; AST/ALT ≤ twice upper normal) unless due to metastatic disease

No significant pulmonary symptoms, adequate pulmonary function including diffusing capacity ≥ 50% normal

Adequate cardiac function (ejection fraction ≥ 50% by NUCLEAR GATED CARDIAC scan)

For patients with estrogen receptor positive tumors (≥ 10 fmol/ml) are eligible if the disease has progressed after at least one hormonal manipulation

Patients with estrogen receptor positive tumors and rapidly progressive tumor are not required to have had prior hormonal therapy

Patients with estrogen receptor negative (or unknown) status are eligible

Adequate pre-transplant bone marrow biopsies with sufficient hematopoiesis to permit engraftment.

Total dose of Adriamycin (or equivalent) ≤ 450 mg/m^2

No history of malignant neoplasm aside from breast cancer except for patients treated curatively for basal or squamous cell carcinoma of the skin or carcinoma in situ of the cervix, or patients who have lived without relapse more than ten years since definitive therapy for any other malignancy

Seronegativity for HIV virus, no overt evidence of AIDS

Patients must not be pregnant or lactating

No evidence of CNS disease of any etiology

Attainment of at least a partial response to induction chemotherapy

Appendix 5. Northeastern Ontario Regional Cancer Centre Eligibility Criteria for High-Dose Chemotherapy/Autologous Hematopoietic Stem Cell Transplantation

Female patients with histologically proven metastatic breast cancer

Untreated with chemotherapy for metastatic disease. If prior adjuvant chemotherapy, must have been completed 6 months prior to entry onto study

Age 18-55 years

Patients with estrogen receptor positive tumors (≥ 10 fmol/ml) are eligible if the disease has progressed after at least one hormonal manipulation. Such patients are also eligible if there is evidence of life threatening disease which cannot await a trial of hormonal manipulation

Patients with estrogen receptor negative tumors (<10 fmol/ml) are eligible if the disease has progressed after at least one hormonal manipulation

ECOG performance status ≤ 2

Measurable or evaluable lesions

Normal bone marrow, liver, renal, and cardiac function as defined by:

White blood cell count ≥ 3.5 x 10^9/L

Absolute neutrophil count ≥ 1.5 x 10^9/L;

Platelet count ≥ 100 x 10^9/L

Hemoglobin ≥ 100 x 10^9/L

Bilirubin ≤ 20 mmol/L

AST and ALT ≤ 2.5 x upper normal limit, or ≤ 4 x upper normal limit if liver metastases present

Serum creatinine ≤ 126 mmol/L

No uncontrolled or significant cardiac disease (myocardial infarction less than one year preceding, congestive heart failure of any degree, any history of cardiac arrhythmias, any history of second or third degree heart block)

Adequate cardiac function (ejection fraction ≥ 50% by NUCLEAR GATED CARDIAC scan) If prior anthracyclines, must have been < 300 mg/m^2 doxorubicin, < 400 mg/m^2 epirubicin, < 100 mg/m^2 mitoxantrone

Adequate pulmonary function (vital capacity, DL_{CO} > 75% predicted for age)

Ability to give signed informed consent

No serious or uncontrolled systemic illness (investigator's discretion)

No evidence of CNS involvement by breast cancer

If prior radiation, must have been to less than 25% of bone marrow bearing areas

No other concomitant chemotherapy or immunotherapy

No prior history of malignant neoplasm aside from breast cancer except for patients treated curatively for basal or squamous cell carcinoma of the skin or carcinoma in situ of the cervix

Seronegativity for HIV virus

Females of childbearing age must have negative serum or urine pregnancy test results obtained within 2 days of initiation of treatment. Patients must not be pregnant or lactating

Attainment of at least a partial response to induction chemotherapy

Appendix 6. Eligibility Criteria for Control Patients

Patients must have a histologically documented diagnosis of breast carcinoma with evidence of recurrent or metastatic disease.

Patients must be between 18 and 60 years of age

Patients may have received prior systemic adjuvant therapy (chemotherapy or hormonal therapy)

ER positive patients may have received prior hormonal manipulation for metastatic disease

Patients with ER negative or ER unknown tumors are eligible.

Patients with rapidly progressive visceral disease are eligible.

Patients must have an estimated ECOG performance status of 0-2

Patients must have adequate end organ function:

Serum creatinine < 120 umol/L (1.5 mg/dl).

Serum bilirubin < 26 mmol/L (2 mg/dl), AST, ALT < 2x normal unless due to metastatic disease.

White blood cell count $\geq 3.5 \times 10^9$/L

Absolute neutrophil count $\geq 1.5 \times 10^9$/L;

Platelet count $\geq 100 \times 10^9$/L

Hemoglobin $\geq 100 \times 10^9$/L

No known significant abnormalities of pulmonary function:

Chronic bronchitis or emphysema by history
No abnormalities suggesting chronic pulmonary disease on chest radiograph
Diffusing capacity if known not less than 50% of normal).

No known significant abnormalities of cardiac function:

History of myocardial infarction which has impaired left ventricular function
Unstable angina
History of arrhythmias
Left ventricular ejection fraction if known $\geq$ 50%
If left ventricular function unknown, must have normal cardiac silhouette

Any other medical problems (hypertension, diabetes) must be adequately controlled.

Patients would have been deemed able to provide informed consent.

Patients must demonstrate an estimated partial response to 3 cycles of chemotherapy

If prior anthracyclines, patients must have received a total dose of adriamycin of ≤ 450 mg/m^2 or epirubicin ≤ 800 mg/m^2

Patients with a history of malignant neoplasm aside from breast cancer are ineligible except for patients treated curatively for basal or squamous cell carcinomas of the skin or carcinoma of the cervix in situ, or who have lived without relapse from any other curatively treated malignancy for more than ten years.

Patients who are HIV seropositive or who have clinical evidence of AIDS are ineligible.

Patients with known CNS involvement (parenchymal or leptomeningeal) are ineligible.

Patients who are pregnant or lactating are ineligible.

Appendix 7. Metastatic Breast Cancer: Conventional Therapy Datasheet

Name: OCF: DOB:

Eligibility:

Age 18-60 at dx of metastases

Histologic confirmation of primary disease

patient captured at 1st chemo for metastatic disease

ECOG 0-1

Creat ≤120

Bili ≤ 26 unless known to be secondary to mets

PFT's: no hx COPD, no COPD/ILD by CXR unless abnormal is malig related

LVEF ≥ 50%, no sig CAD (hx MI, angina, CHF)

Hematologic parameters (ANC > 1500, plats > 100)

Other significant/chronic medical problems

Ineligibility:

doxorubicin > 450mg /m2 epirubicin > 800/m2

other malig (except basal/squame skin/CIN, or curative malig < 10 yr)

known HIV/AIDS

known CNS metastases

Name: OCF: DOB:

Path Date Initial Dx: Breast: L R Histo: CIS ID IL MC IC

T: 0 1 2 3 4 N: 0 1 2 # pos nodes: M: 0 1

Stage: I II III IV Staging confirmed:

ER value: PR value:

Primary Therapy: Mastectomy Lumpectomy/Radiation Axilla Rads: Y N

Systemic Adj Therapy: Tamoxifen start stop

Chemotherapy (regimen) start stop

# of cycles

Date of recurrence: by imaging or bx

Sites of Recurrence: liver lung bone nodal locoregional skin

Metastatic Therapy1: start stop reason

Metastatic Therapy2: start stop reason

Metastatic Therapy3: start stop reason

Metastatic Therapy4: start stop reason

Date 1st chemo for mets: regimen cycles response

2nd chemo for mets: regimen cycles response

2nd chemo for mets: regimen cycles response

4th chemo for mets: regimen cycles response

5th chemo for mets: regimen cycles response

date 1st documented progression: or last known FLUP:

date death or last known FLUP:

Appendix 8. Variable List and Definitions for Final Common Database

1. **Pt:** Patient Subject Number (1-289)

2. **Centre** : Treatment Centre

   Ott = Ottawa

   Sud = Sudbury

   Oma = Omaha

3. **Rx** : Treatment Group

   1= CCT

   2 = HDCT/AHPCT

4. **Age at Diagnosis:** Age in years at initial diagnosis of breast cancer

5. **Breast:** Site of Initial Breast Cancer:

   1 = Left

   2 = Right

   3 = Bilateral

6.**# pos:** Number of histologically positive lymph nodes at diagnosis.

7. **Stage:**Stage of Disease at Initial Diagnosis

1= I

2= IIA

3= IIB

4= IIIA

5= IIIB

6= IV (metastatic at initial diagnosis)

8. **ERP:** Estrogen receptor status

0 = negative (receptor value < 10 pmol/g tissue)

1 = positive (receptor value ≥ 10 pmol/g tissue

9. **PRP:** Progesterone receptor status

0 = negative (receptor value < 10 pmol/g tissue)

1 = positive (receptor value ≥ 10 pmol/g tissue)

10. **Adj_Rads:** Use of adjuvant radiotherapy to breast ± locoregional nodes

0 = no radiation therapy received

1= radiation therapy received

11. **Adj_Chem:** Use of adjuvant chemotherapy

0 = no adjuvant chemotherapy received

1 = received non-anthracycline based adjuvant chemotherapy

2= received anthracycline based chemotherapy

12. **Adj_Horm**: Use of adjuvant hormonal therapy

0 = no hormone therapy received

1= hormone therapy received

13. **DFI**: Disease-free interval (months)

14. **Age_BMT**: Age at beginning of systemic chemotherapy

15. **Met_horm**: Use of hormonal therapy for metastatic disease

0 = no hormone therapy received

1= hormone therapy received

16. **nmethorm**: Number of different metastatic hormonal therapies received

17. **nmetchem**: Number of different metastatic chemotherapy regimens received

18.**bone1***: Bone metastases at initial recurrence

0= no bone metastases

1= bone metastases

19. **liver1***: Liver metastases at initial recurrence

0= no liver metastases

1= liver metastases

20. **lung1***: Lung metastases at initial recurrence

0= no lung metastases

1 = lung metastases

21. **pleura1***: Pleural metastases (nodules or pleural effusions) at initial recurrence

0= no pleural metastases

1 = pleural metastases

22. **skin1***: Skin metastases at initial recurrence

0= no skin metastases

1= skin metastases

23. **locoregion1***: axillary node/breast/chest wall recurrence/metastases at initial recurrence

0= no locoregional disease

1= locoregional disease

24. **no_sites1***: Number of organ sites of metastases at time of initial recurrence

25. **visceral1***: Presence of visceral (liver, lung etc) metastases at initial recurrence

0= no visceral disease

1= visceral disease

26.**bone2**: Bone metastases at time of chemotherapy/high-dose therapy

0= no bone metastases

1= bone metastases

27. **liver2:** Liver metastases at time of chemotherapy/high-dose therapy

0= no liver metastases

1= liver metastases

28. **lung2:** Lung metastases at time if chemotherapy/high-dose therapy

0= no lung metastases

1 = lung metastases

29. **pleura2:** Pleural metastases (nodules or pleural effusions) at time of chemotherapy/high-dose therapy

0= no pleural metastases

1 = pleural metastases

30. **skin2:** Skin metastases at time of chemotherapy/high-dose therapy

0= no skin metastases

1= skin metastases

31. **locoregion2:** locoregional (axillary node, breast) recurrence/metastases at time of chemotherapy/high-dose therapy

0= no locoregional disease

1= locoregional disease

32. **no_sites2:** Number of organ sites of metastases at time of chemotherapy/high-dose therapy

33. **visceral2:** Presence of visceral (liver, lung etc.) metastases at time of chemotherapy/high-dose therapy

0= no visceral disease

1= visceral disease

34. **statuspre:** response/remission status after chemotherapy

0= stable disease after chemotherapy

1= partial response after chemotherapy

2= complete response after chemotherapy

3= no evidence of disease (disease resected)

35. **Marrow@BMT:** Presence of bone marrow metastases at time of high-dose chemotherapy

0= no bone marrow metastases

1= bone marrow metastases

36. **cond:** High-dose chemotherapy (conditioning) regimen

1= CTH (cyclophosphamide, thiotepa, hydroxyurea)

2=CMCb (cyclophosphamide, mitoxantrone, carboplatin)

3= CMVb (cyclophosphamide, mitoxantrone, vinblastine)

4= CMTx (cyclophosphamide, mitoxantrone, paclitaxel)

5= CTCb (cyclophosphamide, thiotepa, carboplatin)

37. **PROD_T:** Progenitor cell product infused

1= bone marrow

2= peripheral blood progenitor cells

3= combination marrow/peripheral blood progenitor cells

38. **CenOS:** censor value for overall survival

0= alive

1= dead

39. **OS:** value for survival duration (initial metastases to death/last follow-up) in months

40. **OSBMT:** value for survival duration after transplantation (transplantation to death/last follow-up) in months

41. **cenprog:** censor value for progression

0= no progression

1= progression

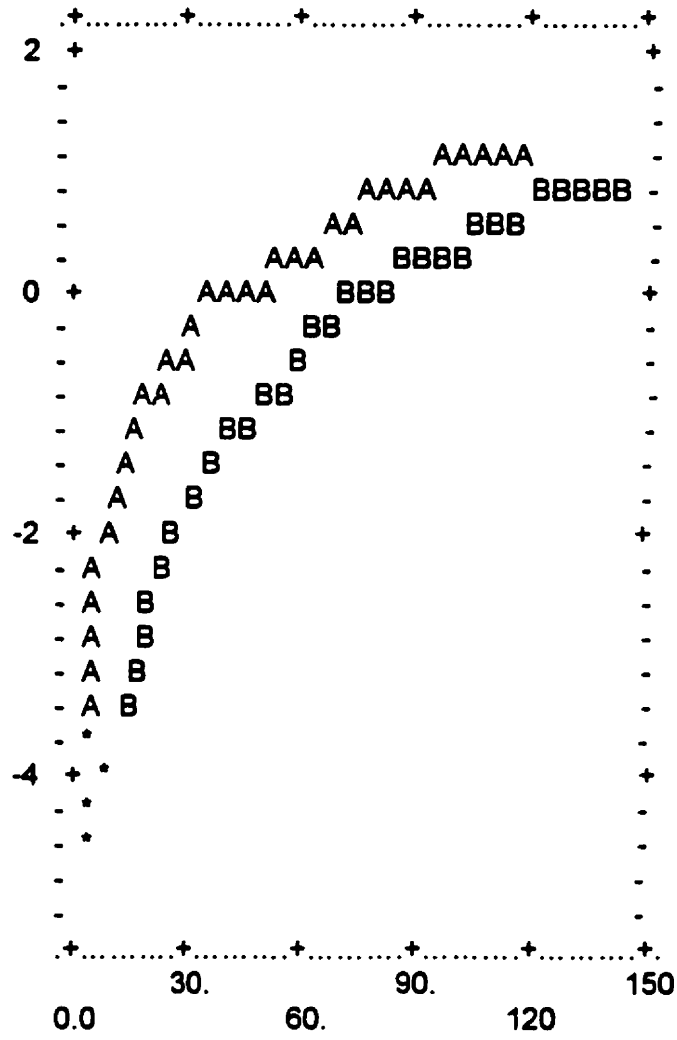
42. **prrecchemo:** duration of time from initial metastases to initial chemotherapy in months

43. **progrche:** duration of time from chemotherapy to first post-chemotherapy progression in months

44. **progrbmt:** duration of time from progenitor cell transplantation to progression in months

(* = data only available for patients from Omaha, Ottawa)

Appendix 9. Log Minus Log Plot of Estrogen Receptor Status and Overall Survival



A = Estrogen receptor negative
B= Estrogen receptor positive