Role of Autologous Bone Marrow Transplantation in the Treatment of Human Malignancies

Internal Medicine Grand Rounds

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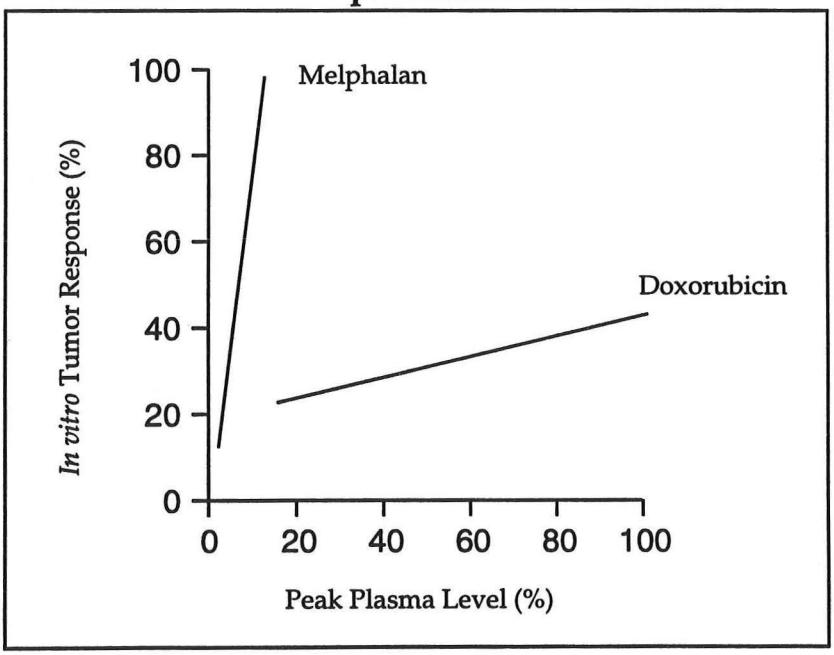
A. Rationale for Autologous Bone Marrow Transplantation

The rationale for intensive high-dose chemotherapy and autologous bone marrow transplantation is based on the fact that a steep dose-response relationship exists between the amount of chemotherapy administered and the fraction of tumor cells killed (1). For a given malignancy, the slope of such curves varies with different treatment regimens. Chemotherapeutic regimens that utilize autologous bone marrow transplantation should have bone marrow suppression as their dose-limiting toxicity. The strategy for autologous bone marrow transplantation rests with the expectation that escalating the dose of chemotherapy from conventional levels to those likely to require bone marrow support result in a higher level of tumor kill to be curative. Hematopoietic recovery is ensured by infusing into either the patient bone marrow, or more commonly, peripheral blood stem cells that have been previously harvested and cryopreserved. The increase in the dose of chemotherapeutic agents leading to the requirement for marrow support before reaching other severe toxicities is not as high as might be anticipated. However, since a portion of the dose-response relationship is frequently linear-log, a relatively small increase in dose may result in a large increase in tumor kill (2). Most dose-response curves have been established for single agents such as cyclophosphamide (3), melphalan (2), and total body irradiation (4). Figure 1 shows a steep response between the dose of melphalan dose and its ability to kill lymphoma cells. In contrast, increasing the dose of doxorubricin to similar levels results in only a small increase of killing these cells.

Dose-Response Relationship for Lymphoma Treated With Melphalan or Doxorubicin



Alkylating agents are the primary agents used for intensive chemotherapy regimens for several reasons. These agents show a steep dose-response curve for many hematologic and other malignancies. For most of these agents, the dose limiting toxicity is marrow suppression. There is not significant cross-resistance between different alkylating agents such that combinations of these drugs, such as carmustine and melphalan (5) or cyclophosphamide, cisplatin, and carmustine (19) are frequently used in protocols combining high-dose chemotherapy and autologous bone marrow transplantation.

Dose-Limiting Toxicity Associated With Alkylating Agent Use

Drug	Dose-Limiting toxicity associated with standard dose therapy	Dose-Limiting toxicity associated with therapy utilizing bone marrow support
Cyclophosphamide	Myelosuppression	Hemorrhagic myocarditis
Cisplatin	Myelosuppression	Nephrotoxicity, neurotoxicity
Carmustine	Myelosuppression	Pulmonary fibrosis, toxic hepatitis
Melphalan	Myelosuppression	Mucositis
Busulfan	Myelosuppression	Anorexia, veno-occlusive disease, autoimmune disease
Thiotepa	Myelosuppression	Mucositis, CNS syndromes

Dose Escalation of Single Agents

Agent	Usual Conventional Dose	Maximum Dose without ABMT	Maximum Dose with ABMT	Limiting Extramedullary Toxicity
Cyclophosphamide mg/kg	50	200	200	Cardiac
Carmustine mg/m^2	200	600	1200	Pulmonary, hepatic
Melphalan mg/m^2	40	120	200	Gastrointestinal, hepatic
Etoposide mg/m^2	360	1200	2400	Gastrointestinal
Ifosfamide mg/m^2	5000	8000	18,000	Renal, bladder
Thiotepa mg/m^2	50	180	1135	CNS, renal
Carboplatin mg/m^2	400	1600	2000	Hepatic, renal
Total body irradiation cGy		350	1400	Pulmonry, hepatic

For most agents the myeloablative dose is rarely more than three-fold the maximum tolerated dose (6) without hematopoietic rescue. Table compares conventional and high-doses of single chemotherapeutic agents with levels requiring autologous bone marrow transplantation (7, 8). Of the single chemotherapy agents investigated, thiotepa appears to demonstrate the highest dose escalation which is five-fold over the maximal tolerated dose without marrow support. Surprisingly, agents such as melphalan can be escalated by less than two-fold before toxicity to sites other than the bone marrow is seen. However, combinations of different chemotherapeutic can give steep dose-response curves with a relatively small dose escalation in one or more of these agents translating into an additional log in tumor cell killing agents. Thus combinations of different chemotherapeutic agents that would normally be myelotoxic when used in conjunction with autologous bone

marrow transplantation result in acceptable toxicity and higher response rates than seen with conventional chemotherapy.

B. Comparison of Autologous and Allogeneic Bone Marrow Transplantation

Autologous transplantation allows patients to receive a high-dose of myelosuppressive chemotherapy followed by infusion of their own hematopoietic cells to restore marrow function. The hematopoietic cells contain stem cells that proliferate and differentiate into mature blood lineage, such as leukocytes, platelets, and erythrocytes. Stem cells can be collected from bone marrow or peripheral blood. Standard-dose combination chemotherapy can be given before autologous transplantation to reduce the tumor burden. The long-term outcome of transplantation is better in patients who have a minimum volume of disease that responds to standard-dose chemotherapy. For transplants, bone marrow is collected from the posterior superior iliac crests by multiple aspirations while the patient is anesthetized. Marrow collection should occur at a time when the marrow is normally cellular and does not contain malignant cells. With current techniques, marrow cryopreservation can be reliably performed, and the stored cells can remain viable for more than 5 years. Peripheral blood stem cells are collected by apheresis, usually by using a large-bore vascular catheter in the subclavian vein. Leukapheresis is repeated by using continuous-flow cell separation. To collect an adequate cell dose for transplantation, 5 to 10 daily leukaphereses are required. Circulating progenitor cells are mobilized to a higher level during the recovery phase following chemotherapy and treatment with granulocyte colony-stimulating factor (G-CSF, filgrastim (Neupogen).

Autologous vs Allogeneic Transplantation

Treatment	Autologous	Allogeneic
HDCT	Yes	Yes
GVHD	No	Yes
Graft failure	Rare	Yes
Relapse	Higher	Lower
Treatment Mortality	Less than 10%	10% to 40%
Cost	Lower	Higher

The dose of antineoplastic agents or radiation that can be administered clinically is limited by its toxic effects to normal tissues. Bone marrow suppression is the dose-limiting toxicity for most chemotherapeutic agents. The doses of radiation and many drugs can be substantially escalated to more effective levels if followed by transplantation of normal hematopoietic cells, thus rescuing the patient from severe and prolonged myelosuppression. For dose-intensive therapy to be successful, the neoplasm must exhibit

a dose-dependent response to chemotherapy and/or irradiation so that one (or possibly several) course of intensive combined-modality treatment can eradicate the malignant cells. The actual preparative regimen involves chemotherapeutic drugs alone or combined with radiotherapy. After the preparative regimens are administered, supportive care is required to deal with complications related to prolonged neutropenia and the toxic effects of the preparative regiments.

The marrow cells or peripheral blood stem cells are infused intravenously after completion of the preparative regimens. The cells circulate transiently, and sufficient numbers of stem cells home to the marrow and restore hematopoiesis. Peripheral blood counts are profoundly suppressed as a result of the effects of the conditioning treatment but generally recover within three to four weeks with marrow transplantation and within 2 to 3 weeks with peripheral stem cell transplantation. Patients generally receive G-CSF, to accelerate marrow recovery until the absolute neutrophil count is greater than 1,000/mm^3.

Comparison of Autologous Bone Marrow vs Peripheral Blood Stem Cell Transplantation

Source	Bone Marrow	Peripheral-blood progenitor cells
Method of Collection	Multiple aspiration of illac crests	Aphereisis via subclavian catheter
General Anesthesia	Yes	No
Duration for Collection	One Day	One to several days
Complication	Pain, anesthesia related, rare nerve damage	Vascular access-related pneumothorax, infection
Recovery of WBCs to > 500/ml	2 weeks	2 weeks
Platelets > 100,000	4 to 5 weeks	2 to 3 weeks
Tumor contamination	Yes	Yes

Autologous transplantation is less likely to produce major side effects than is allogeneic transplantation because the infused cells are not subject to rejection and do not mediate graft-vs-host disease. There is also no immune-mediated graft-vs-tumor effect from the donated cells. Therefore, the most frequent cause of treatment in failure in autologous transplantation is recurrence of the underlying malignancy. In contrast to autologous bone marrow harvest, peripheral blood stem cells offer an alternative source of hematopoietic cells for transplantation. This is an effective approach for patients who cannot undergo marrow harvest, such as patients who have undergone pelvic radiotherapy. Peripheral blood stem cells are also a potential source for patients with marrow and malignant cell involvement, although the significance of contaminating malignant cells in the peripheral blood remains to be determined. Transplantation of large numbers of stem cells results in more rapid recovery of platelets than does autologous marrow transplantation. Thus infusion of peripheral blood stem cells in conjunction with high-dose chemotherapy can be an effective modality to treat selected human malignancies.

Major Controversies in Autologous Transplantation

Autologous vs allogeneic transplantation

Blood-progenitor cells derived from bone marrow vs peripheral blood

Optimal dose-intensive preparation regimens

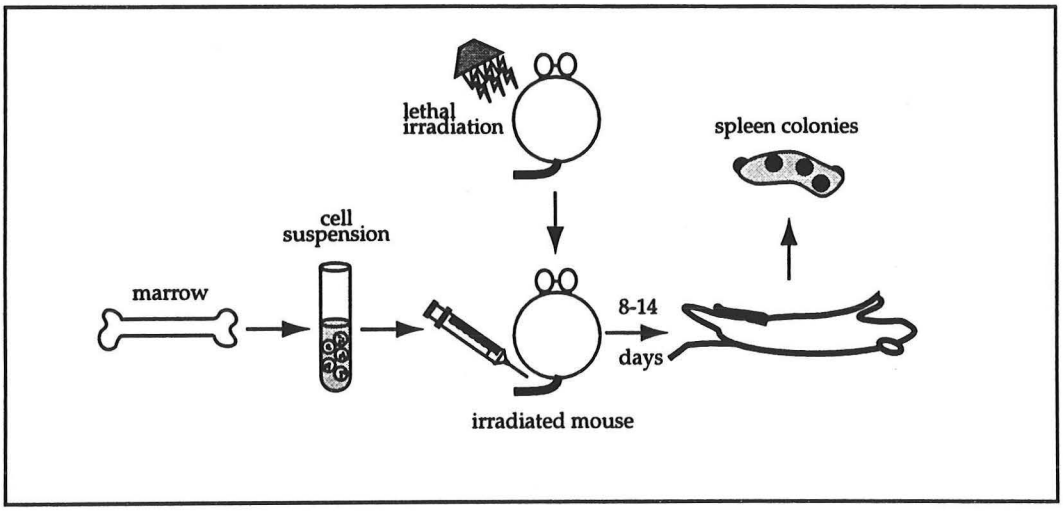
Role of purging

Improvement in supportive care

C. Stem Cell Biology

Stem cells are the progenitors of both hematopoietic components and lymphocytes (9). They can proliferate and differentiate into cells of myelomonocytic, erythroid, megakaryocytic and lymphoid lineages while retaining the capacity to maintain long-term hematopoiesis by a process of self-renewal (10-13). An adequate number of stem cells is necessary for marrow engraftment after either autologous marrow or peripheral stem cell rescue. Stem cells normally comprise 1-2% of cells in the bone marrow and about 0.1% of nucleated peripheral blood cells (14). They are identified and quantitated using either functional assays or immunophenotyping. Several *in vitro* clonogenic assays have been developed for the quantification of human hematopoietic progenitors. Lineage committed stem cells such as colony-forming unit granulocyte/monocyte (CFU-GM) can be assayed in semisolid culture media. Assays for more primitive stem cells have been developed by culturing them with irradiated long-term culture-initiating cells, but these assays are more complex and will not be described here (15).

Identification of Pluripotent Stem Cells



The CFU-GM assay can be used to measure the progenitor cell function in peripheral blood stem cell harvests and this assay correlates well with neutrophil recovery after peripheral stem cell transplantation (16). The disadvantage of this assay method is the length of time (2 weeks) needed to get useful information about stem cell quantity and quality. Alternatively, the amount of CD34 that is present on the surface of progenitor cells can serve as a good marker of stem cells. CD34 is a highly glycosolated membrane glycoprotein whose expression is limited to hematopoietic precursors and vascular endothelial cells (36). Virtually all colony-forming cells are included in the 1.5% of bone marrow cells which are CD34 positive (17). This cell population is heterogeneous, with high CD34 expression in the more primitive cells that lack expression of lineage-associated antigens (14, 18). CD34 expression diminishes as lineage-associated antigens, such as CD33 (myeloid), Tdt and CD7 (T lymphoid) and Tdt, CD10 and CD19 (B lymphoid), are expressed (14). CD34 surface expression correlates with CFU-GM assays and can be used to predict the time to marrow regeneration following transplantation of stem cells (19, 20). Purified CD34 positive cells that lack the coexpression of lineage-associated antigens can produce multiple colony forming cells when cultured *in vitro* with marrow stromal cells. In addition, highly purified CD34 positive cells are able to produce stable hematopoietic reconstitution when infused after supralethal myeloablative therapy of either baboons or humans.

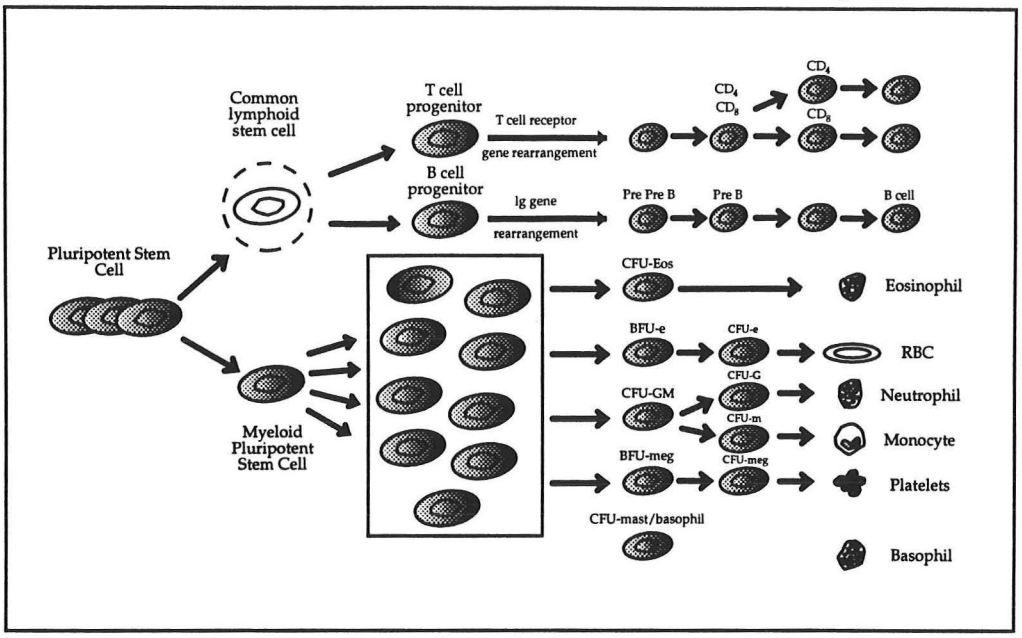
All the bone marrow cells expressing the CD34 antigen are not the actual pluripotent hematopoietic progenitors, since both primitive and more committed progenitors coexpress this antigen. Thus pluripotent cells are a subset of the total CD34 positive cell pool. Additional efforts are currently underway to better define immunophenotyping of this cell subpopulation. Results from these studies indicate that the more immature CD34 positive progenitor cells coexpress low levels of Thy- antigen but not CD38, CD5, CD10, CD33, CD71 and HLA-DR-antigens (21, 22). The immature CD34 positive cells represent

Stem Cell Phenotype

No hemopoeitic lineage-specific markers
Sca^{+} kit^{+}
$CD34^{+}$ KIT^{+}
Rhodamine dull
$CD38^{-}$ Dr^{-} $CD34^{+}$
Quiescent or low
percentage of cells in S phase

approximately 5 to 20% of the total CD34 positive marrow population depending on the techniques used to analyze these cells (23-25). Multicolor flow cytometry has allowed the detection of small numbers of CD34 positive cells in the peripheral blood (26). Bone marrow and blood-derived hematopoietic pools are in dynamic equilibrium dependent on age and other circumstances. At birth, the human umbilical cord blood contains rates of CD34 positive cells similar to those observed in normal adult bone marrow and consequently can be considered an alternative source of cells for hematopoietic transplantation (25, 27). In normal adults, CD34 positive cells are present in the blood at no more than 10% of bone marrow or umbilical cord blood concentrations (22). However, after mobilization with high-dose chemotherapy and recombinant human hematopoietic growth factor G-CSF, the peak of circulating CD34 positive cells can reach levels up to 1,000 time higher than normal even exceeding the numbers that can be harvested by multiple bone marrow aspirations (28).

Stem Cell Progenitors Reconstitute Hematopoiesis



Whether peripheral blood CD34 positive cells are equivalent to their marrow counterparts remains somewhat questionable. *In vitro* colony forming assays have shown that peripheral blood CD34 positive cells have the same capacity as bone marrow CD34 positive cells to form unilineage (CFU-GM, BFU-E, CFU-Meg), multilineage (CFU-mix) or blast cell colonies in *in vitro* colony forming assays (29). CD34 positive cells which do not coexpress CD38 antigen, and are considered to be very immature hematopoietic progenitors, are present in two to three times lower levels in leukapheresis products (6% of the total CD34 positive pool) than in umbilical cord blood (12%) and in bone marrow (19%) (23, 30). These data suggest that in addition to more committed progenitors, very immature hematopoietic cells are present in lower proportions indicating somewhat decreased proliferative capacity as compared to bone marrow (31).

Experience has shown rapid, complete, and sustained hematopoietic reconstitution capacity of hematopoietic lineages if adequate numbers of peripheral blood stem cells are infused (32, 33). Because most peripheral blood stem cell transplants have been autologous, long-term repopulation has not been genetically proven in humans, although such evidence exists in gene-marked autologous and allogeneic animal models (34). For example, Kiem *et al.* have shown the presence of marked CFU-GM, granulocytes 48 to 75 weeks after autologous BCT in a dog model (35). Several laboratories have initiated studies to mark mobilized blood cells genetically and to follow expression of the marked genes following autologous BCT in humans.

D. Stem Cell Mobilization Techniques

The use of cytokines, such as G-CSF, GM-CSF and interleukin 3 (IL-3), to mobilize CD34 positive cells demonstrated that G-CSF increases CFU-GM by a factor of 56, GM-CSF by 20 and IL-3 by only 10 (36-38). The rise in peripheral CD34 counts is adversely affected by marrow infiltration and intensive chemotherapy pre-treatment (39-41).

Hematopoietic Growth Factors

Growth Factor	Bioactivity
GM-CSF	Granulocyte and macrophage colony formation
G-CSF	Granulocyte colony formation and differentation factor
Erythropoietin	Red cell formation
IL-1	B- and T-cell regulator
IL-2	T-cell growth factor.
IL-3	Stimulates granulocyte, macrophage, eosinophil, mast cell and megakaryocyte colony formation
IL-4	B-cell proliferation and immunoglobulin secretion
IL-5	B-cell differentiation and immunoglobulin secretion
IL-6	B-cell differentiation and immunoglobulin secretion
IL-7	Stimulation of pre-B cell production
IL-9	Erythroid colony formation
IL-10	Inhibits cytokine synthesis by T cells; increases cytotoxic T cell number and function
IL-11	B-cell, megakaryocyte, and stem cell stimulator
IL-12	Natural killer (NK) cell stimulator
IL-13	Similar to IL-4

G-CSF can be used successfully to mobilize stem cells on its own, but is associated with a less reproducible peak in peripheral stem cell numbers and with a smaller stem cell yield (41). However, this mobilization approach has been successfully used in allogeneic peripheral stem cell transplants, where chemotherapy cannot be given to healthy donors (42).

Planned collection schedules for peripheral blood stem cells were developed in the early 1990s using a single dose of cyclophosphamide with peak circulating stem cells occurring 16 days later (43). The stem cell collection could be augmented by the use of daily G-CSF after chemotherapy increasing its yield by a factor of more than 250 (44). One mobilization schedule used a combination of cyclophosphamide 1.5 grams/m^2 and daily G-CSF 10 μg/kg. This regimen resulted in mild post-chemotherapy pancytopenia and a reproducible peak in peripheral stem cell numbers at about day 10 when the peripheral leucocyte count is greater than 8 x 10^9/l (45). This method gave sufficient stem cells in several aphereses treatments for subsequent marrow engraftment in most patients. The adequacy of the stem cell collection following apheresis was determined by measuring CFU-GM, which should be greater than 50 x 10^4/kg and the CD34 count which should be greater than 0.5-5 x 10^6/kg.

Stem cells can be mobilized efficiently into the blood by chemotherapy or cytokines, requiring only one to three aphereses. The effect of administration of peripheral blood stem cells to patients treated with high-dose chemotherapy results in enhanced neutrophil and platelet recovery and reductions in febrile days coupled with shorter

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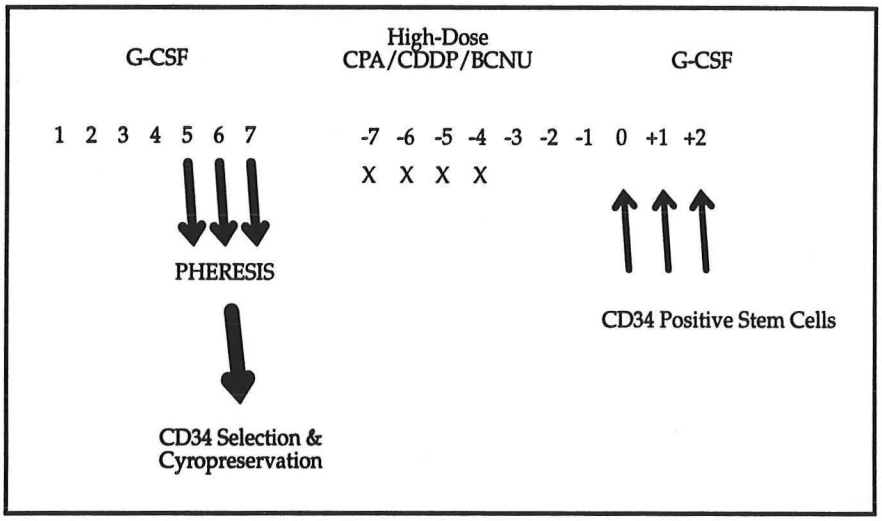
hospitalizations (46). These advantages have resulted in increasing use of mobilized peripheral blood stem cells at the expense of autologous and allogeneic bone marrow transplantation. The optimal method of blood cell mobilization is not yet defined. Some generalizations are possible, however, despite the limited availability of comparative data. There is considerable heterogeneity in mobilized stem cells which may result in blood cell yields below engraftment threshold. Among comparable G-CSF-mobilized adult patients, the progenitor yield may vary up to 200- fold (47). Prior large-field radiotherapy significantly reduces progenitor yield with an average reduction of $1.8x10^6$/kg CD34 positive cells, whereas previous treatment with chemotherapy causes a smaller reduction (48).

Schematic for G-CSF Mobilization of Stem Cells

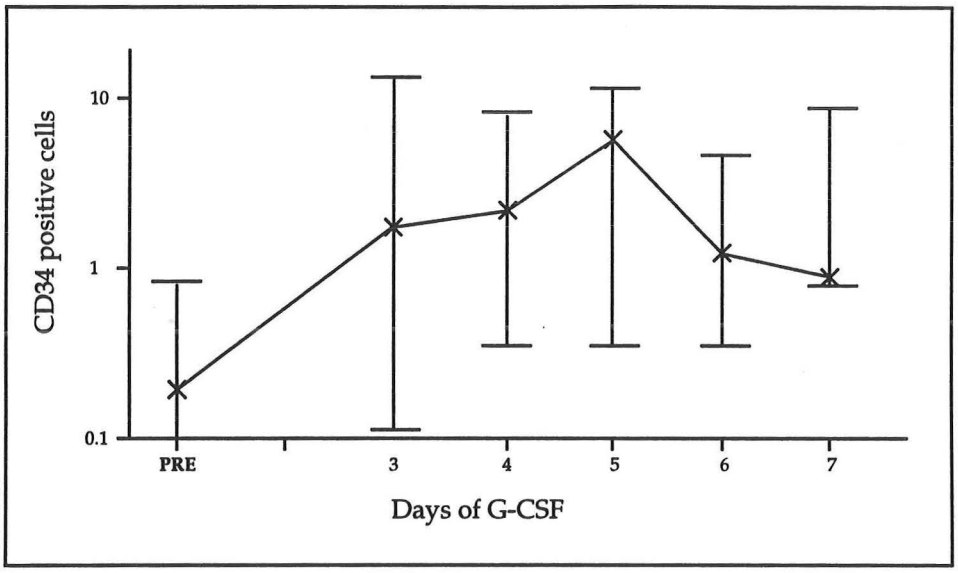


Presumably, this result relates to chemotherapy and radiotherapy-induced hematopoietic stem cell damage. Chemotherapy may also cause microenvironmental or stromal cell damage because stem cell reconstitution of hematopoiesis was progressively slower after three cycles of high-dose therapy than with one cycle of high-dose chemotherapy despite identical blood cell numbers on each occasion (49). It is important to define an adequate or threshold stem cell dose that leads to predictable, rapid, and sustained hematopoietic reconstitution. Progenitor cell assays using CFU-GM and the number of CD34 positive cells have increasingly replaced the nucleated cell yield. Hematopoietic reconstitution can be predicted by both these measurements, although there are laboratory and patient variables making the relationship complex. Blood cell rescue with 20 to $50x10^4$ CFU-GM/kg body weight or $2x10^6$ CD34 positive cells/kg body weight gives rapid reconstitution and a threshold of 5 to $20x10^4$ CFU-GM/kg body weight or 0.5 to $2x10^6$ CD34 positive cells/kg body weight can give sustained but not rapid, reconstitution of hematopoiesis (50).

Mobilization by chemotherapy alone now probably has no application. Progenitor cell yields are at best approximately comparable to those obtained with cytokine mobilization alone and significantly inferior to combined chemotherapy and cytokines (51-57). High-doses of myelosuppressive drugs to mobilize stem cells results in significant

morbidity and even mortality due to pancytopenia (58). Cytokine mobilization alone is effective, and with G-CSF there is essentially no morbidity apart from mild bone pain. Previously G-CSF or GM-CSF have been used exclusively, but cytokines acting on early multipotent stem cells, such as interleukin-3, SCF and Flt-3 in combination or sequentially with G-CSF or GM-CSF are being evaluated (59, 60). It is too early to draw definitive conclusions regarding their efficacy but they probably enhance progenitor yield. Cytokine mobilization affords no tumor cytoreduction, and it is applicable only when tumor control is stable and sustained. It is probable that cytokine mobilization alone will have its major application in allogeneic blood cell transplantation and procurement of blood cells for gene therapy. Combined chemotherapy and cytokine mobilization is the method of choice for cancer patients. It allows tumor cytoreduction while allowing the collection of hematopoietic cells (61). It is likely that less myelotoxic or standard-dose chemotherapy combined with cytokines will produce adequate progenitor yields with reduced morbidity since a low neutrophil nadir is not a prerequisite for progenitor release (55).

Concentration of Peripheral Blood CD34 Positive Cells in Patients Receiving G-CSF



The flow cytometry assay for CD34 positive cells has the advantage of providing results in about two hours (62). It may permit the selection of the best timing to initiate the apheresis procedure and to predict the total number of hematopoietic progenitor cells collected by each leukapheresis. The CD34 positive cell content in the peripheral blood should be measured starting at the seventh to tenth day after the onset of chemotherapy. It is important to check the peripheral blood daily from this date for two main reasons (63-65). First, it is likely that the first CD34 cells to appear are the most immature cells which are responsible for restoring long-term hematopoiesis. Second, the peak of CD34 positive cells in the circulation does not last long and must be recognized as soon as possible so that an early start of the apheresis procedure can allow maximal collection of these very immature cells. Data obtained from each collection allow apheresis to be continued only until the preestablished required threshold of CD34 positive cells and consequently CFU-GM doses are obtained. Careful monitoring such as this permits collection of sufficient numbers of both primitive and committed progenitor cells to ensure rapid and stable engraftment from only one to three aphereses.

Several models of blood cell separators are presently on the market for apheresis. They can be divided into two main categories: semicontinuous or continuous flow blood cell separators (66). The latter are undoubtedly the best performing and also the most comfortable for the patients. They are generally automatic and computerized. Two or three blood volumes are generally processed during each apheresis session, which takes an average of three to five hours. The procedure is generally repeated two to five times (depending on the mobilization protocol) on consecutive days, except if the condition of the patient is too weak. Each blood stem cell collection is processed to reduce the volume so as to reduce the amount of the cryoprotectant (DMSO) required, and to remove as much as possible of mature red cells, granulocytes and platelets which are unnecessary for hematopoietic reconstitution but are responsible for clinical toxicities (67). Plastic bags containing stem cells are then cryopreserved, and stored in liquid nitrogen until reinfusion (68). Before reinfusion of the stem cells typically performed 24-48 hours after completing the pre-transplant conditioning regimen, the patient is prepared with a diuretic regimen to avoid hypervolemia related to the re-infusion of an average of six to ten blood stem cell units. Each cryopreserved bag is thawed quickly in a 37°C water-bath at the patient's bedside prior to infusion and is infused rapidly through a peripheral venous catheter in less than 15 minutes. The reinfusion of peripheral blood stem cells is generally performed within two to three hours.

E. Tumor Cell Contamination of Peripheral Blood Stem Cells

One of the advantages of peripheral blood stem cell transplantation is lower levels of contamination by tumor cells. This premise has not been closely evaluated until recently because sensitive and specific detection assays for contaminating malignant cells were not available. Now a variety of technologies are available to evaluate malignant cells including immunocytochemistry, polymerase chain reaction, fluorescence-activated cell sorting, and clonogenic assays. Only the latter provides evidence of malignant cell viability or whether the cells infused with the stem cells may be involved in subsequent relapse.

Malignant Contamination of Bone Marrow and Peripheral Blood

Disease	Patients	Method	BM % positive	Steady-state PB % positive	Mobilized PB % positive
Breast cancer	48	Immunocytochemistry	62	ND	10
Neuroblastoma	56	Immunocytochemistry	40	25	ND
Breast cancer	9	Immunocytochemistry	56	22	78
Lung cancer	29	Immunocytochemistry	10	7	21
Non-Hodgkin's lymphoma	212	Polymerase chain reaction	100	49	ND

There is increasing evidence that malignant cells contaminate peripheral blood stem cell harvests from patients with neuroblastoma, breast and lung carcinoma, and lymphoma (69-71). Recent data using PCR analysis of bone marrow from patients with breast cancer indicates a much higher incidence of metastases than previously thought (72). Using primers to detect mRNA from the cytokeratin19 gene which is expressed in a variety of

epithelial cells, these authors found that 52% of stage II, 57% of stage II and 80% of stage IV breast cancer were positive using PCR analysis. This is more than double the incidence of metastases determined using immunocytochemistry. Tumor cell contamination is more likely in patients with marrow involvement. In mobilized (69) and steady-state blood cells (71), the incidence and quantity of peripheral blood tumor contamination is significantly lower than in the bone marrow (69, 71). However, peripheral blood stem cell mobilization by combined chemotherapy and G-CSF treatment results in an increase in blood tumor cell contamination compared with steady state peripheral blood levels (70). Tumor cell presence was investigated using immunocytochemical assays in steady-state and mobilized blood cells and in bone marrow in 46 patients with stage II-IV breast and lung cancer (70). Following mobilization, an additional 21% of patients had detectable tumor cells, including all stage IV breast cancer and 50% of small cell lung cancer patients. Patients having tumor cells in the steady state peripheral blood had substantially higher levels of circulating tumor cells following mobilization. A two- phase pattern of tumor cell mobilization was noted. Between days 1 to 7 of chemotherapy, tumor cells were seen in patients without marrow involvement, suggesting mobilization of extramedullary disease. During the usual period of stem cell collection, between days 9 to 16, only patients with marrow disease showed tumor mobilization. Although tumor cells could be recruited into the circulation in patients without detectable marrow involvement, this was considerably less common than in those with involvement (69, 70).

Methods of Marrow Purging in Autologous Bone Marrow Transplantation

A. Immunologic
   - Monoclonal antibodies and complement
   - Immunotoxins
   - Monoclonal antibodies and magnetic microspheres

B. Biophysical
   - Photoactive agents

C. Culture
   - Long-term bone marrow culture

D. Positive selection for normal early progenitors
   - Enrichment for CD34 + cells

It is unclear whether different mobilization methods will result in different levels of tumor contamination. Further investigation is required including methods of quantitative detection. The mechanisms of tumor cell recruitment are unknown. Contaminating tumor cells in the peripheral blood stem cells may reflect higher tumor load in the patient and therefore a poorer prognosis, irrespective of the infusion of tumor cells. However, gene marking studies in acute myeloid leukemia suggest that reinfusion of malignant cells at least contributes to relapse (73). Routine purging of tumor cells from blood cell collections cannot yet he recommended, but there is evidence at least in autologous BMT that this may reduce the risk of relapse in lymphoma (74).

The presence of tumor cells in peripheral blood stem cells and their potential for inducing disease relapse is a critical question to address so as to better understand the clinical situations where this procedure is efficacious. For example, patients with relapsed or refractory intermediate or high-grade non-Hodgkin's lymphoma (NHL) are incurable with standard therapy. However, a number of patients who might benefit from high-dose therapy cannot have this treatment because of inability to obtain hematopoietic stem cells from the bone marrow that are not contaminated by tumor. Peripheral blood stem cell transplants can offer a potential solution to this problem (75). In NHL as in other malignant diseases, blood-derived stem cells have been hypothesized to be less contaminated by residual tumor cells than bone marrow. Data which seem to confirm this hypothesis at least partially have been reported (76). Occult tumor cell detection by culture techniques was systematically performed on peripheral blood stem cells and on bone marrow harvested at the same time in patients with NHL in remission. Residual bone marrow involvement by lymphoma cells was detected in 36% of cases, while peripheral blood harvests were culture-positive only in 5% of cases. Among patients without bone marrow involvement fewer relapses were observed in those who received peripheral blood stem cells than in those having received autologous bone marrow. Patients with bone marrow involvement did significantly better following peripheral stem cell transplants than recipients with either culture-positive or culture-negative bone marrow who underwent autologous bone marrow transplants (76). These results strongly suggest that peripheral blood cells have a lower likelihood of tumor contamination than bone marrow cells, and/ or that blood cells may have an antitumor effect which is not achieved with bone marrow cells. Thus it seems likely that peripheral blood stem cells might have a lower proportion of tumor contamination compared with bone marrow harvested at the same time.

Results of Autologous Transplantation for Treatment of Malignancy

Disease	Benefit of autologous transplantation	Benefit of allogeneic transplantation	Long-term DFS of autologous transplantation
AML	Yes	Yes	30% to 40%
ALL	Yes/No	Yes	30% to 40%
CLL	Unknown	Yes	Unknown
CML	Promising	Yes	Unknown
MDS	No	Yes	Unknown
Lymphoma	Yes	Yes/No	40% to 60%
Myeloma	Yes	Yes	25 to 35%
Hodgkin's Disease	Yes	Yes/No	20% to 60%
Breast Carcinoma	Promising	Unknown	20%, stage IV 75%, stage II-III

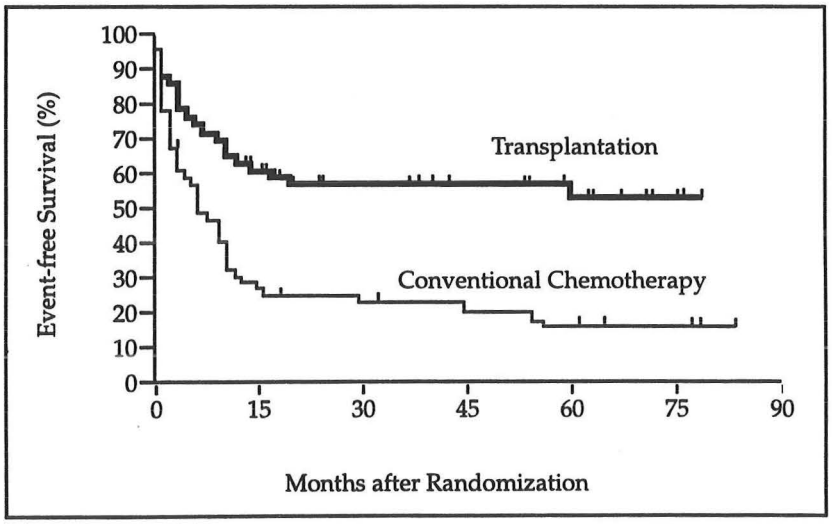
F. Peripheral Blood Stem Cell Transplantation and High-Dose Chemotherapy for Treatment of Hematologic Malignancies

1. Intermediate-grade and High-grade non-Hodgkin's Lymphoma

Intermediate-grade and high-grade non-Hodgkin's lymphomas are usually treated with chemotherapy using various combinations of drugs and patterns of drug scheduling. CHOP chemotherapy is the classical first generation regimen that is used in resulting in a 5-year survival of 30-50% (77). The subsequent use of more complex and intensive drug combinations has not resulted in an improved survival (77, 78). The outcome for patients relapsing after first-line chemotherapy and treated with conventional salvage regimens is extremely poor with fewer than 10% surviving at 3 years (79).

The majority of series of patients treated with high-dose chemotherapy and either autologous bone marrow or peripheral stem cell rescue have either primary resistant or relapsed NHL. In one single centre study, the 5-year actuarial survival and progression-free survival were 41% and 35%, respectively (80), which is similar to other published series (81-83). Another study demonstrated that patients with relapsed chemosensitive non-Hodgkin's lymphoma have a better outcome when treated with high-dose chemotherapy and autologous bone marrow transplantation than those treated with conventional chemotherapy (84). There was a 5-year overall survival and progression-free survival of 53% and 45% for the transplant group vs. 32% and 12% for the nontransplant group (84). Patients with chemosensitive disease do considerably better, with a 5-year actuarial survival varying from 40% to 60% compared to 0% to 30% for those with chemoresistant disease. A case-controlled study performed by the European Blood and Marrow Transplant group (EBMT) showed that outcome in high grade NHL after autologous bone marrow transplantation and peripheral blood stem cell therapy was similar (85).

Survival of Patients with Relapsed Lymphoma by Autologous Transplantation or Conventional Chemotherapy



The role of myeloablative chemotherapy as a first-line treatment of high grade NHL remains unclear. No difference was found in the overall survival at 4 years in patients with

a partial response to three cycles of CHOP chemotherapy who were randomized to receive either five additional courses of CHOP or cyclophosphamide and total body irradiation in conjunction with autologous bone marrow rescue (86). In poor prognosis patients with NHL who achieved a complete response to either conventional chemotherapy or high-dose chemotherapy with autologous bone marrow transplant, the 3-year survival was similar for both groups at 69% for the autologous bone marrow transplant group and 71 % for the chemotherapy group (87). There was no clear survival advantage for patients receiving a transplant in first complete remission. However, a recent study in patients with aggressive B cell lymphomas with bulky extramodel disease demonstrated a survival rate of 76% for patients treated with high-dose chemotherapy and autologous transplantation versus a 49% survival rate chemotherapy with MACOP-B (88). Thus, high-dose chemotherapy with either ABMT or PBSCT rescue can be recommended in patients with relapsed, chemosensitive non-Hodgkin's lymphoma and potentially in a subset of poor prognosis patients on initial presentation. However, its use as first-line therapy in patients with slowly responding disease has not been demonstrated.

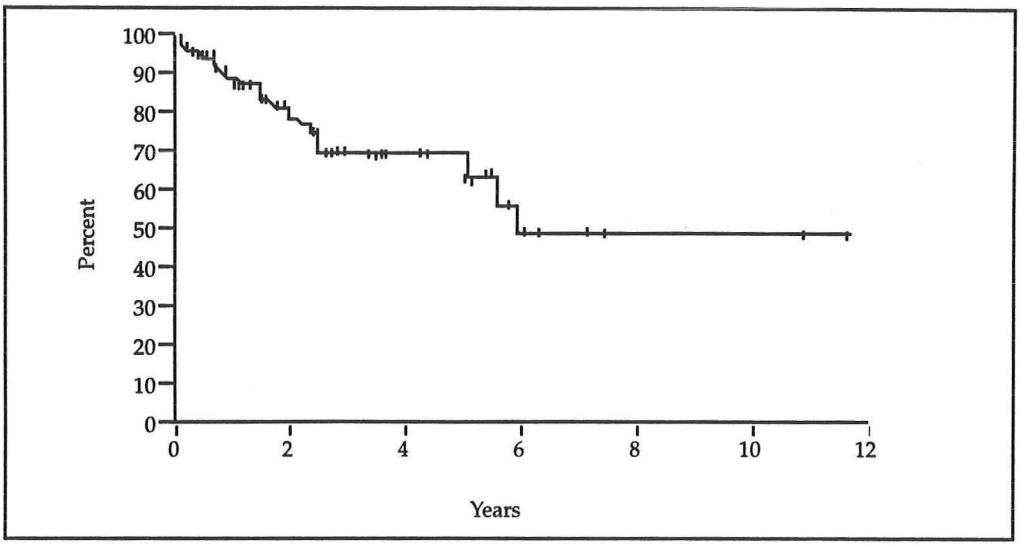
2. *Low-grade non-Hodgkin's Lymphomas*

Low-grade non-Hodgkin's lymphoma is incurable with conventional treatment, but can be managed for many years with intermittent doses of oral alkylating agents and local radiotherapy (89). Fifty per cent of these patients will be alive with disease 10 years after diagnosis. This outcome may be acceptable in an elderly population, but a more intensive approach with curative potential is warranted in younger patients. The Dana-Farber Cancer Institute, and St Bartholomew's Hospital in London have treated patients with follicular lymphoma in radiological remission and no evidence of marrow infiltration with myeloablative total body irradiation and cyclophosphamide followed by autologous bone marrow transplants (89, 90). The mononuclear cell fraction isolate from the autologous bone marrow harvest was purged and assayed for minimal residual disease using the polymerase chain reaction (91). Patients tolerated the treatment well and the relapse rate at 2 years was lower than expected using historical controls. Patients in complete remission did better in the Dana-Farber group (91), but no difference in outcome was found between patients with and without residual disease in the London group. This difference may be attributable to different purging methods in the two centres. The Dana-Farber group used a cocktail of three antibodies and immunomagnetic beads, whereas the St Bartholomew's group used a single antibody and complement-mediated lysis (91, 92). A recent study also demonstrated the feasibility of high-dose chemotherapy and autologous transplantation in patients with low grade lymphoma. There was a 65% overall survival at 4-years and a 44% failure free survival but no evidence of a plateau in survival (93). Thus prolonged failure free survival can be achieved but is unclear whether patients are cured or survival is prolonged (93).

Low grade lymphoma is associated with marrow infiltration in a significant number of cases, therefore the value of marrow purging becomes an important issue. A European study is attempting to address this issue. Patient recruitment has been difficult because most groups now favour the use of peripheral blood stem cells versus autologous bone marrow (94). Selection of stem cells based on their CD34 positive immunophenotype has been advocated as an alternative way of removing tumour cells. The efficacy of this approach is unproven as the follicular lymphoma associated translocation t(14;18) occurs during immunoglobulin gene rearrangement in lymphoid committed stem cells which express CD34. The clinical indications for using high-dose therapy in low-grade lymphomas remains unclear, as long-term follow-up will be needed to show a survival advantage in this group of indolent lymphomas. In addition, the value of purging or the

use of CD34 positive stem cell transplants in this context needs to be studied in a randomized fashion.

Survival Following Autologous Bone Marrow or Peripheral Blood Stem Cell Transplantation for Low-Grade Lymphoma



3. Hodgkin's Disease

Conventional treatment of Hodgkin's disease with radiotherapy, chemotherapy or both results in a 5-year survival of 70% (95, 96). A worse prognosis is associated with extensive disease, B symptoms and some histological subtypes. Up to 55% of patients relapsing more than a year after first-line therapy can be salvaged with more intensive treatment (97, 98) using alternating chemotherapy regimens. The outcome for the remaining patients and for those who relapse a second time remains poor with conventional salvage treatment.

The 30% of patients with Hodgkin's disease not cured with first-line treatment fall into three groups: those with primary refractory disease, those relapsing less than a year after treatment and those relapsing more than a year after treatment. The difference in prognosis of these groups is illustrated by a single-centre study of 155 cases, which included patients with primary refractory disease and those relapsing less than a year after completing treatment (99). The patients were treated with the myeloablative BEAM chemotherapeutic regimen (carmustine, etoposide, cytosine arabinoside and melphalan) followed by autologous bone marrow rescue. The overall and progression-free survival at 5 years was 55% and 50%, respectively, for this group of poor prognosis patients. Patients with primary refractory disease fared worse (33% progression-free survival) than the relapsed patients (44-70% depending on the number of previous relapses). Similar results have been obtained by other groups using different conditioning regimens and either autologous bone marrow transplant or peripheral stem cell rescue (100-104).

The combined results of various series using high-dose treatment with either autologous bone marrow or peripheral blood stem cells have allowed recommendations about which patients may benefit from this treatment (105). These include failure to achieve complete remission with appropriate first-line chemotherapy, relapse within 6

months of finishing treatment, relapse after second-line treatment and first relapse after induction treatment with alternating schedules, such as MOPP/ABVD. Studies are in progress to investigate the role of high-dose therapy in patients with Hodgkin's disease in first remission with bad prognosis disease (106, 107). The main disadvantage with this approach is the difficulty in reliably identifying patients with a poor prognosis, leading to concern that a significant proportion of patients will be over-treated.

Due to bone marrow abnormalities, not all patients with relapsed Hodgkin's disease who are eligible for high-dose chemotherapy and peripheral blood stem cell transplants are candidates. Bone marrow hypocellularity may result from either earlier radiation therapy or from prior chemotherapy or both. Patients with relapsed or refractory Hodgkin's disease with bone marrow abnormalities have been treated with high-dose therapy and autologous bone marrow transplantation in a trial by Kessinger et al. (108). Following transplantation of unmobilized stem cells, 34 out of 68 evaluable patients achieved CR and 24 achieved a partial response. At 46 months, the actuarial progression-free survival was 33% for all patients. Patients with marrow disease due to involvement with Hodgkin's disease had a statistically worse actuarial free-survival than patients who underwent transplants for other reasons. Similar results were achieved in another series enrolling 28 patients with relapsed Hodgkin's disease who were chemosensitive and were treated with BEAM chemotherapy in conjunction with peripheral blood stem cell transplants (109). These results compared favorably to patients who received high-dose chemotherapy and autologous bone marrow transplants. This data suggests that high-dose chemotherapy in conjunction with peripheral blood stem cell or autologous bone marrow transplant is effective therapy for patients with Hodgkin's disease having primary resistant second relapses.

4. Chronic Lymphocytic Leukemia

Chronic lymphocytic leukemia (CLL) is an indolent disease characterized by an abnormal proliferation in most cases of monoclonal B lymphocytes in the bone marrow and lymphoid tissues (110). The clinical course of the disease is extremely variable: some patients have a normal life span, whereas others die within a few years after diagnosis depending on the presence of poor prognostic factors at the start of the disease. The prognostic features are usually characterized by the Rai-Binet stages. Usually treatment for patients with CLL is initiated at Binet stage B-C or Rai stage III-IV, or when there are signs of progressive disease or poor prognostic factors (111). The rationale for this therapeutic strategy is that patients in earlier stages of the disease tend to have a higher response rate than those at an advanced stage. Optimal management of CLL is still questionable. At present, there are no proven cures for CLL, but the natural history of the disease and the advanced age of many patients makes prolongation of survival a reasonable therapeutic goal in most cases.

The traditional approach to the management of CLL is based on the activity of alkylating agents and corticosteroids. Patients resistant to these agents are treated with nucleoside analogs although some authors report better clinical results using these compounds in untreated patients. These drugs have been established as effective agents in the majority of cases of CLL (112). However, patients ultimately relapse and the choice of salvage therapy by conventional means does not offer a cure. Most patients at diagnosis are over 60 years of age and 90% are over 50 years of age thus the introduction of more aggressive protocols aimed at obtaining long disease-free survival is necessarily limited to a low number of cases. However, in younger patients with poor prognosis factors, the management of this disease might substantially alter in the near future, with alternative and innovative approaches aimed at achieving cure or long disease-free survival. One of the promising treatments is high-dose therapy followed by hematopoietic stem cell rescue.

One of the biological characteristics of CLL is that the marrow is invariably infiltrated with a high number of leukemic cells; the conventional therapies are not able to induce minimal residual disease in the marrow, so the definition of complete remission is quite different from that of acute leukemia and is largely clinical rather than biological. Khoury et al (113) reported the results of autologous bone marrow transplantation in 13 patients with advanced CLL whose disease progressed after treatment with fludarabine-based therapy. The marrow harvest was performed when malignant cells in the bone marrow biopsy constituted less than 15% of the total cells present. The preparative regimen was cyclophosphamide and total body irradiation and the marrow was purged of cyclophosphamide-resistant lymphocytes with immunomagnetic separation. Nevertheless, the majority of patients had detectable leukemia cells after the *in vitro* treatment. All patients who underwent transplantation had a major response and nine had a complete remission. Those with advanced resistant disease relapsed within 1 year of the transplant and two cases with more than 15% lymphocytes in their marrow at the time of transplant remain in remission at 15 and 17 months post-BMT (113).

The Dana Farber Cancer Institute (114) compared the efficacy and toxicity in patients treated with autologous and allogeneic bone marrow transplantation for 32 poor prognosis CLL patients with < 10% bone marrow involvement. The patients underwent autologous bone marrow transplants with marrow purged with anti-B-cell monoclonal antibodies. The pre-transplant regimen was cytoxan and total body irradiation and 26 patients were alive and disease-free at a median follow-up of 12 months after the graft. The predicted disease-free survival at 18 months was 74%. The results in a group of 13 CLL patients with the same characteristics treated with allogeneic bone marrow transplantation were similar and no statistically significant difference was demonstrated in overall disease free survival between allogeneic or autologous bone marrow transplant. Although there was a high rate of opportunistic infections in patients with allogeneic transplants.

Data pooled data from the European Blood and Marrow and International Bone Marrow Transplantation Registries on the outcome of allogeneic transplantation (70 cases) and autologous (29 cases) stem cell transplants in B-CLL treated between 1984- 1995 was reported (115). In the peripheral stem cell transplants group, all patients received chemotherapy with or without fludarabine prior to transplant and 69% of cases were in complete remission at the time of transplantation. The conditioning regimens included cytoxan and total body irradiation or high-dose chemotherapy without irradiation (BEAM and other regimens). The overall complete response rate post- transplant was 83%, but it was only 27% in the patients with residual disease at the time of transplant with 79% of patients alive a median of 36 months post-graft. The three-year survival probability was 48% for allogeneic bone marrow transplant vs 42% for autologous bone marrow transplant demonstrating no long-term differences between the treatments.

The experience with high-dose chemotherapy and autologous bone marrow transplantation for patients with CLL is extremely limited due to a several factors such as a long-term survival with conventional therapy in a good percent of patients, the advanced age of most patients and the difficulty in obtaining minimal residual disease in the bone marrow, which is required for the success of autologous bone marrow transplantation. The use of immunoglobulin gene rearrangement and the polymerase chain reaction for detection of residual disease demonstrates that the majority of patients in complete remission have residual leukemic cells present. Thus, purging of stem cells before their reinfusion is likely indicated in this disease. The introduction of new therapeutic agents, such purine analogs such as fludarabine which are highly effective in achieving higher response rates. permits consideration of high-dose therapy with stem-cell rescue as an innovative and feasible strategy in younger patients with poor prognosis CLL. The toxicity

of the treatment is acceptable and should not preclude further use of this approach in poor prognosis CLL patients.

5. Multiple Myeloma

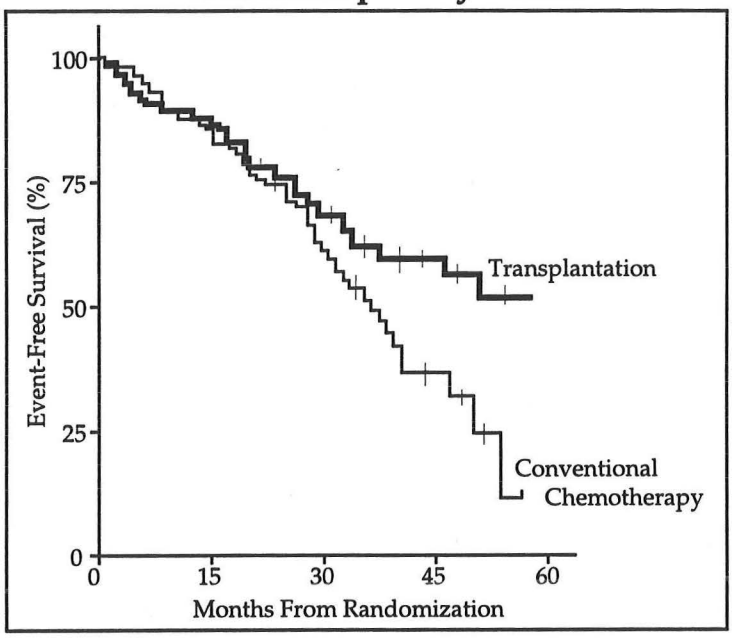
Multiple myeloma is an incurable B-cell malignancy afflicting mainly elderly people with a median age of 65 years (116). A combination of oral melphalan and prednisone has been the standard treatment for almost 30 years (117). Although more than one-half of newly diagnosed patients will respond to standard therapy, only approximately 5% will attain complete remission (118, 119). Poor prognostic factors include patients with high B-2 microglobulin, C-reactive protein, plasma labeling index, chromosomal abnormalities (11q and 13q) and IgA myeloma (120). Median survival ranges from less than 1 year to 5 years, with an average of 30-36 months in most larger series. Altering the melphalan/prednisone regimen by the addition of alkylating agents and anthracyclines has not extended the median survival beyond 3 years (118). The low incidence of complete remission is probably a reflection of marked drug resistance of myeloma cells even at diagnosis resulting from genetic alterations (120).

Until recently, patients with multiple myeloma were not considered for intensive dose regimens because of their usually advanced age and frequently brittle clinical condition (121). However, the utilization of peripheral blood stem cells and hematopoietic growth factors has substantially reduced the duration of marrow aplasia and transplant-related mortality so that high-dose therapy can be performed safely in patients up to age 70 (122-125). Recent data suggests that tandem or triple transplants can enhance the ability to achieve a complete response to high-dose chemotherapy (116). Allogeneic bone marrow transplantation from matched sibling donors appears to be the most promising approach toward cure but is limited to only about 7% of all patients because of age restrictions and donor limitations as well as considerable procedure-related morbidity and mortality (126).

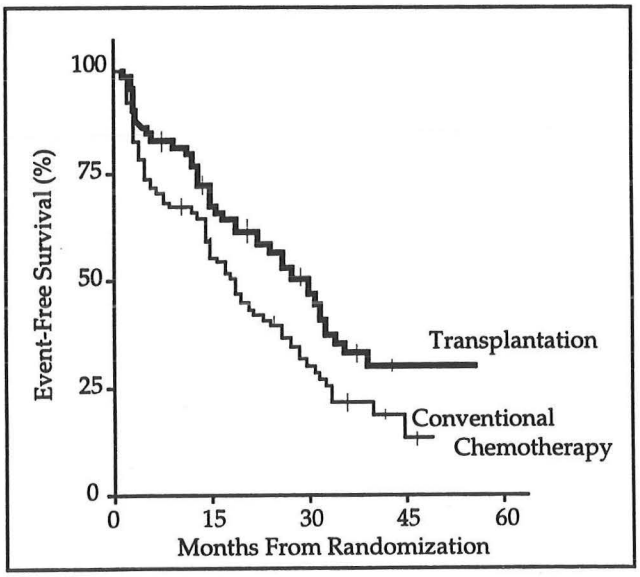
In myeloma patients undergoing peripheral blood stem cell transplants, the period of treatment with standard therapy with alkylating agents should be limited to less than 12 months to avoid stem cell damage (127) and an increased risk of treatment induced myelodysplastic syndromes (128). For example, $2x10^6$ CD34 positive stem cells/kg were needed for effective reconstitution of hematopoiesis in myeloma patients with less than 24 months of standard chemotherapy while $5x10^6$ CD34 positive stem cells/kg were needed for reconstitution in patients received more than 24 months of standard chemotherapy (127). In older patients treated with extensive prior chemotherapy where additional chemotherapy cannot be used to mobilize stem cells, G-CSF (16 μg/kg) alone could be used to mobilize sufficient stem cells for successful autologous transplants (129). Since multiple myeloma is a disease of terminally differentiated B-cells with low proliferative capacity the presence of up to 30% of plasma cells gave similar remission and survival durations compared to bone marrow with less than 5% plasma cells (130). Although circulating myeloma cells can be detected in the peripheral blood, their effect on the outcome of autologous stem cell transplants has not been defined. The role of autologous stem cell transplants in myeloma patients with renal insufficiency and those older than 60 years of age have also been studied (124, 131). Patients with renal insufficiency (creative 2 mg/dl) are excluded from most autologous transplantation protocols. Since melphalan pharmokinetics are not influenced by renal impairment, myeloma patients with renal insufficiency have been treated with high-dose chemotherapy and autologous stem cell transplants. No differences in toxicities, early death or complete response rates were observed although overall survival was better in patients with normal renal function (131).

Thus patients with renal insufficiency should not be excluded from autotransplant trials. Patients older than 60 years of age have an increased transplant-related mortality (6% vs 1%) and lower rate of complete remission (23% vs 5%) (124). In a multivariate regression analysis, age was not an unfavorable prognostic factor for overall survival. Hematopoietic stem cell reserve was similar in younger and older patients. Thus patients older than 60 with normal organ function and adequate stem cell mobilization should not be excluded from high-dose chemotherapy and stem cell transplantation.

Comparison of Overall Survival in Patients with Multiple Myeloma



Comparison of Disease-Free Survival in Patients with Multiple Myeloma



Over several thousand autologous transplants have been performed worldwide in patients with multiple myeloma. Autotransplants have utilized PBSCs, bone marrow, or both in support of multiple chemotherapeutic regimens containing high-dose melphalan with or without total body irradiation (116). A European multicenter study of autotransplants in multiple myeloma, including 207 patients transplanted at 37 centers (median age 49 years), showed a 46% complete response rate and median duration of event free survival and overall survival of 29 and 32 months, respectively (125). On multivariate analysis, sensitive disease, younger age (<45 years), and conditioning with melphalan were identified as independent favorable parameters (125, 132). This data was confirmed in a French study in which high-dose chemotherapy and autologous bone marrow transplantation resulted in a 52% five-year estimated survival versus 12% in myeloma patients treated with conventional chemotherapy (133). These data confirm that high-dose melphalan in conjunction with autologous transplantation is a successful regiment for the treatment of myeloma.

The optimal timing of autotransplantation remains unclear. Autotransplantation was clearly beneficial in patients with primary unresponsive disease with prolongation of median survival from 37 months with standard therapy to 83 months with autotransplantation (116, 133). In contrast to other reports that myeloablative therapy is of limited value for late myeloma and does not benefit patients with sensitive disease early in their disease course (134, 135), results from the University of Arkansas demonstrate that all myeloma patients benefit from this approach (116, 133, 134). Fifty-four percent of patients received standard therapy in excess of 12 months; the complete response rate was 28% with median event free survival and overall survival of 21 and 42 months from transplant, respectively, exceeding the results reported in patients treated with conventional chemotherapy (116, 133). Compared to resistant disease, sensitivity to standard therapy conferred a significantly higher complete response rate (46% vs. 14%) and effected prolongation of both event free survival (31 vs. 20 months) and overall survival (48 vs. 30 months). Patients with primary unresponsive disease had much lower complete response rates (11%) and inferior overall survival than those with sensitive disease. These patients, however, fared significantly better than patients with refractory relapse.

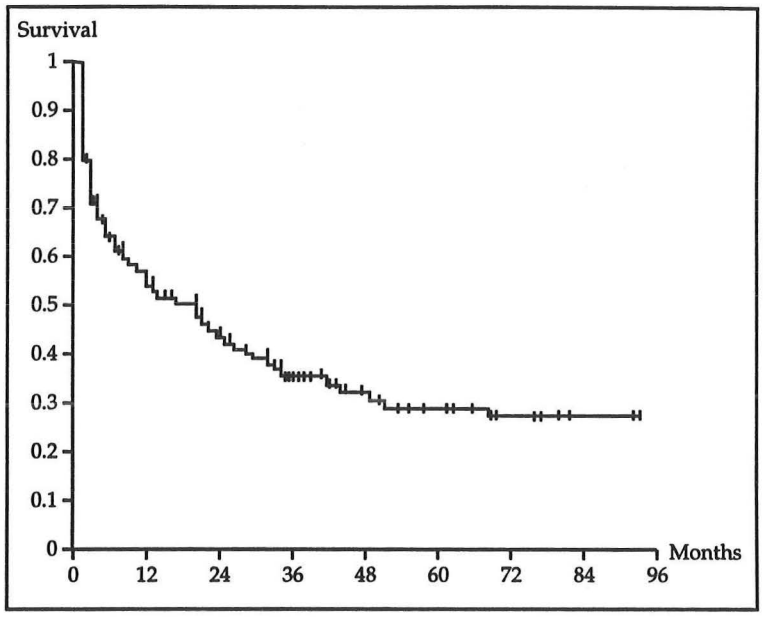
Tandem autologous transplants for patients with multiple myeloma are safe and feasible with approximately 85% of patients expected to complete the planned two cycles of high-dose therapy within 1 year (116, 124). A complete response was achieved in 34% of patients with a transplant-related mortality of 6% within the first year with the median duration of event free survival and overall survival of 27 and 40 months, respectively, from first transplant. The time interval between the two scheduled transplants should not exceed 6 months to avoid tumor regrowth and the likely acquisition of further drug resistance.

Thus clinical trial results of high-dose therapy with autotransplantation in multiple myeloma are gradually maturing and permit the following conclusions: myeloablative therapy can be safely administered in patients up to and exceeding age 70 years with a procedure-related mortality of less than 5% and true complete response can be obtained in approximately 40-50% of newly diagnosed patients. The collective experience with high-dose therapy in multiple myeloma indicates that drug resistance to standard-dose chemotherapy, characteristic of myeloma cells even at diagnosis, can be overcome by dose intensification, which may be further improved with tandem transplants. Although complete response rates have improved with high-dose therapy, plateaus are not yet apparent for event free survival after autologous transplants whereas this appears to be the case after allogeneic transplants. The observation of relapses following autologous transplants has stimulated additional comparative trials of standard versus myeloablative therapy.

Clinical Trials of Allogenic Bone Marrow Transplantation for Multiple Myeloma

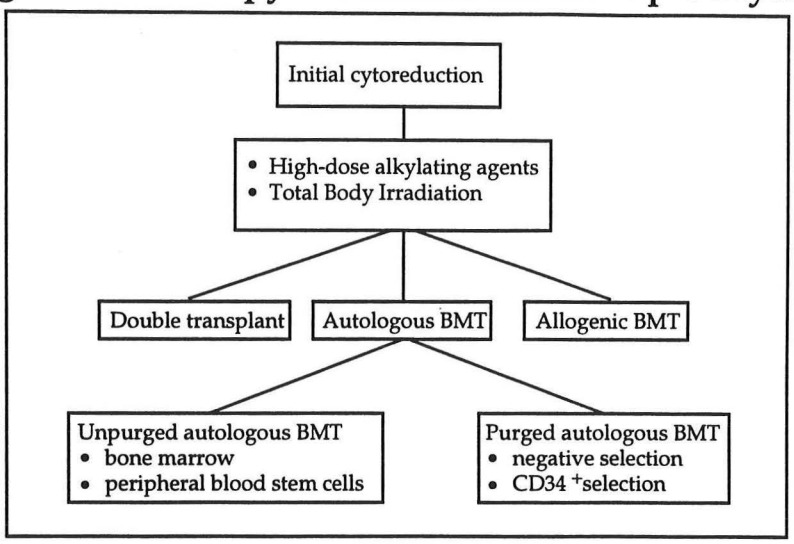
Study	No.	CR (%)	Treatment Mortality(%)	Overall Survival
Gahrton et al	162	44	40	28% at 7 years
Bensinger et al	80	36	42	24% at 4.5 years
Reece et al	26	50	19	46% at 3 years
Anderson et al	14	45	15	NR
Vesole et al	31	41	19	24% at 2 years

Survival in Patients Treated With Allogeneic BMT For Multiple Myeloma



Allogeneic bone marrow transplantation has the advantage of absence of potential tumor cell contamination in the graft. Although the transplant-related mortality is extremely high, approaching 40-50% in the first year post-transplant, a plateau at approximately 30% has been demonstrated for those patients achieving complete response, which has not been observed with autologous transplantation, indicating that cures may occur in an otherwise incurable disease (126, 133). The presence of a graft-versus-tumor effect has been well established in leukemia, particularly in CML (136). Recent results with donor buffy coat infusions in patients with relapsed CML following allogeneic transplant provide direct evidence of a graft-versus-tumor effect in the eradication of hematological malignancies (137). Similar results have been observed in patients with persistent multiple myeloma after T-cell-depleted matched unrelated transplants infused with donor peripheral blood mononuclear cells (138). This antimyeloma effect resulted in resolution of bone marrow plasmocytosis, however, was associated with severe GVHD. Allogeneic BMT could be performed on patients with myeloma at a stage when first-line treatment fails, if the patient is unresponsive to first-line treatment or in patients with poor prognostic factors for conventional chemotherapy such as stage III multiple myeloma, a high ß2-microglobulin level, and IgA myeloma.

High-dose Therapy Modalities for Multiple Myeloma



G. Peripheral Blood Stem Cell Transplantation and High-Dose Chemotherapy for Treatment of Solid Tumors

Studies of animal and human malignancies have quantitatively demonstrated that drug resistance appears to be relative to the dose employed and that absolute resistance is an unusual event. *In vitro* cell culture and animal studies have demonstrated that cytotoxicity for resistant subpopulations appears to be relative and that it is possible to overcome this effect by dose escalation (139-142). Clinical studies have confirmed a steep dose response for the treatment of a variety of solid tumors. The implication is that a one- to twofold dose escalation may have a profound impact on therapeutic efficacy. If the administered drug dose is increased, it tumor-killing ability is also increased, and hopefully, the chance for cure is improved (142). With alkylating agents, the primary class of drugs used to treat solid tumors, this effect continues to be seen over several log increases in drug dose. Furthermore, the slope of the killing curve among various tumor cell subpopulations remains constant and linear once a threshold had been reached. The clinical dose-limiting toxicity of most alkylating agents is myelosuppression. When ameliorated with a bone marrow transplant, a three- to ten-fold increase above conventional doses can often be reached before serious nonhematologic toxicity develops. Thus there is a good rationale for high-dose chemotherapy in conjunction with autologous bone marrow or stem cell transplantation for the treatment of patients with solid tumors.

High-dose chemotherapy sustained by hematopoietic stem cell rescue is an investigative approach used in the treatment of some solid tumors. In most of the previous clinical trials, the source of hematopoietic stem cells was autologous bone marrow, but the use of blood-derived stem cells is becoming increasingly widespread (9). Considering the potential advantage of using peripheral blood stem cells instead of bone marrow as autologous stem cell support after high-dose therapy in solid tumors, two main types of clinical situations can occur. In the first, patients present with a solid tumor without any metastasis, and no bone marrow involvement, but remain resistant to standard chemotherapy. Salvage high-dose chemotherapy could potentially cure such patients, but this treatment may result in secondary myeloablation. Autologous stem cells can be used in this setting for hematopoietic support. In the second situation, patients present with a solid tumor involvement, particularly neuroblastoma breast cancer, or small cell carcinoma of the

lung with bone marrow. The likelihood of tumor cell contamination is probably lower in the blood than in the bone marrow. For example, Sharp et al., applying culture techniques have recently demonstrated in a series of 56 patients with stage I-III breast cancer that the frequency of tumor- cells in marrow harvests was very high compared with peripheral blood cells harvested at the same time: 42% versus 16% (76). Using immunocytological analysis, Moss et al. have shown in stem cell collections from three different series of patients with advanced breast cancer that circulating tumor cells were detectable in significant amounts at an average incidence of 10% of cases even where there was little to no marrow disease detected (143). Thus potential tumor cell contamination of the stem cells remains a problem in this clinical situation.

Although many patients with solid tumors have certainly been treated worldwide with high-dose therapy followed by infusion of stem cells within the past five to six years, it remains extremely difficult to clearly assess the role of this therapy as compared to autologous bone marrow transplantation in terms of the rates of complete response and disease free survival. Indeed many of the reports are difficult to evaluate for several reasons. Most of the first reports published in the literature focused on the feasibility of the method in terms of hematopoietic recovery following transplantation, while the impact of this procedure on the rate of complete response and disease free survival was not evaluated. Also many series enrolled cancer patients receiving either bone marrow or blood-derived stem cells as hematopoietic support after high-dose salvage therapy without any available comparison between the two regimens. However, data presented in the following sections suggests that high-dose chemotherapy in conjunction with peripheral blood stem cell transplants represents an effective therapeutic approach for the treatment of selected solid tumors.

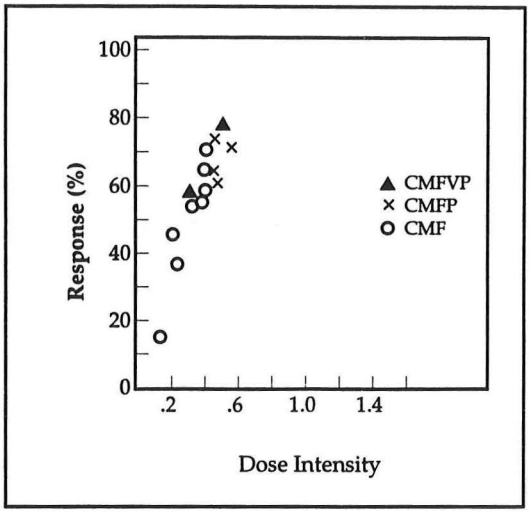
1. Breast cancer

In 1994 there were approximately 183,00 new diagnoses of breast cancer, and 46.300 deaths from the disease (144). Metastatic breast cancer is a lethal disease despite some responsiveness to systemic chemotherapy. Although combination chemotherapy treatment in previously untreated metastatic breast cancer is associated with objective response rates of 40- 80%, the median duration of response is less than a year (145).
The median survival for patients with metastatic breast cancer is only two years; the 5-year survival for patients presenting with metastatic breast cancer is only 19%, and the 10-year survival for such patients is less than 5 %. In addition, at the time of initial diagnosis about 25% of women are considered at high risk for local or distant recurrence despite standard adjuvant therapy because of large tumor size. high number of axillary lymph nodes involved with cancer, and/or poorly differentiated morphologic features at the time of histologic examination. All of these patients could benefit from more effective systemic treatment.

In the 1980s, several groups demonstrated the feasibility of administering high-dose chemotherapy to patients with advanced breast cancer and suggested that such treatment could prolong survival (146-148). Over the past decade, there has been considerable debate over the expected benefit from dose intensification in breast cancer. Some authors suggested a major value (144) and others argue that even moderate changes in dose intensity would be unlikely to yield important differences in outcome (145). The importance of dose intensification in breast cancer therapy was prompted by the successful application of dose intensity in acute leukemia, lymphoma, Hodgkin's disease, neuroblastoma, testicular cancer and other diseases. The use of high-dose chemotherapy,

and autologous bone marrow transplantation (ABMT) represented a logical extension of this concept.

Response Rate vs Dose Intensity in Patients with Breast Cancer



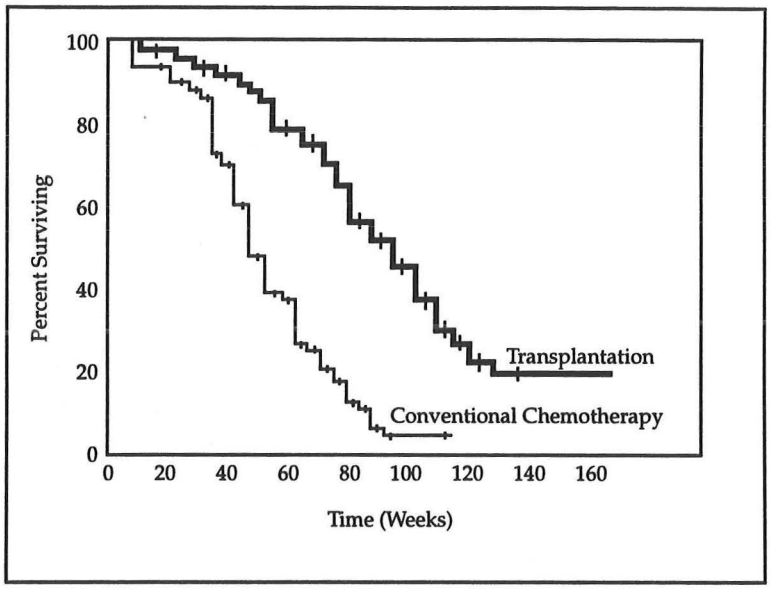
A phase I trial in breast cancer patients conducted by Peters et al defined a schedule and the maximally tolerated doses of a regimen containing cyclophosphamide, cisplatin, and carmustine with autologous bone marrow support (149). Phase II trials have been in sites of prior bulk disease. In the next trial, patients received an AFM (doxorubicin, 5-fluorouracil, and methotrexate) induction regimen, in an attempt to obtain a minimal tumor

Clinical Trials with High-Dose Chemotherapy and ABMT for Metastatic Breast Cancer

Author	Evaluated Patients	Response rate (%)	Complete response (%)
Bouleuc	91	59	18.8
Spitzer	25	95	65
Israel	30	93	40
Peters	22	73	54
Nabholtz	39	75	58
Antman	29	NA	44.8
Grad	23	NA	NA
Vredrenburgh	30	50	7
de Vries	30	NA	NA
Dunphy	58		55
Jones	45		58
Kennedy	30		37
Williams	27		48

burden prior to autologous bone marrow transplantation and high-dose chemotherapy (150). Sixty-eight percent of patients who received three to four cycles of AFM (doxorubicin, 5-fluorouracil, and methotrexate) followed by high-dose chemotherapy achieved complete remission. Approximately 20% of these patients remained free of recurrent disease at a median of 36 months after transplantation. Williams et al. (151), Dunphy et al. (152), Kennedy et al. (153), and Antman and Gale (154) report similar results in stage IV breast cancer patients, with complete remission rates in excess of 65% and unmaintained progression-free survival rates of 20% to 30%. These results are superior to published results with standard therapy. One randomized study in stage IV breast cancer has been performed (155). In this study, patients who received high-dose chemotherapy and autologous bone marrow transplantation had a 51% complete response rate as compared to a 5% complete response for conventional chemotherapy. There was 20% long term survival in patients treated with high-dose chemotherapy as compared to only a 2% for patients treated with conventional chemotherapy.

Survival of Patients Treated with High Dose or Conventional Chemotherapy for Treatment of Metastatic Breast Cancer



A second area where the use of high-dose consolidation may be of major value is in patients who have high-risk primary breast cancer (156-162). Patients who have involvement of 10 or more axillary nodes at the time of initial evaluation suffer a high frequency of relapse despite adjuvant chemotherapy. In most series, only between 20% and 40% of patients remain relapse free at 5 years, with the majority of relapses occurring within the first 2 to 4 years following initiation of therapy (155, 160). Because of this poor prognosis and because the patients are in an optimal complete remission after the surgery, this group might be appropriate for evaluating the effect of high-dose chemotherapy and autologous bone marrow support. These patients have been treated with four cycles of CAF (cyclophosphamide, doxorubicin, fluorouracil) followed by high-dose combination chemotherapy with cyclophosphamide, cisplatin, and carmustine and autologous bone marrow transplantation (161). Eighty patients were analyzed by Kaplan-Meier analysis and found to be progression free and 72% remained event free (relapse or death from toxicity) with a median follow-up of 4 years. This result is far superior to that usually projected for conventional adjuvant treatment, for which 10% to 35% of patients are progression-free at a similar time point (161).

Adjuvant High-Dose Chemotherapy With ABMT for Patients With More Than Four Positive Axillary Nodes

Author	Evaluated Patients	Number of positive axillary nodes	Regimen	Disease Free Survival
Abeloff	53	≥10	Cyclophosphamide/ doxorubicin/methotrexate/ vinchristine/fluorouracin	80% (3 yr)
de Graaf	24	>5	Methotrexate/fluorouracil doxorubicin/vinchristine	84% (5 yr)
Peters	85	>10	Cyclophosphamide/cisplatin/ busulfan	72% (2.5 yr)
Hudis	60	>4	Doxorubicin, cyclophosphamide	84% (15 mo)
Somlo	79	>10	Doxorubicin/etoposide/ cyclophosphamide or cisplatin/ etoposide/cyclophosphamide	NA
Overmoyer	29	>10	Busulfan/cyclophosphamide: cisplatin/cyclophosphamide/ carmustine:cyclophosphamide/ carboplatin/carmustine	85% (3 yr)

Peters and colleagues (163) recently provided an update on the largest experience with high-dose chemotherapy to date on patients with high risk primary breast cancer. After extensive eligibility evaluation, 85 patients initially received six cycles of chemotherapy with standard cyclophosphamide, doxorubicin, and 5-fluorouracil followed by high-dose chemotherapy. Patients were also required to receive irradiation of the chest wall and tamoxifen for 5 years. After a median follow-up of 6 years, the 5-year event-free survival rate was 64% and the overall survival rate was 75% (163). These results were compared with those of historical controls from previous adjuvant chemotherapy trials in which standard-dose chemotherapy was administered. The 5-year event-free survival rate in the controls was 30% to 35%, and the overall survival rate was 37% to 48% (163). This comparison suggests that adjuvant high-dose chemotherapy may be superior to standard-dose chemotherapy for patients with high-risk primary breast cancer (that is, patients in whom more than 10 axillary lymph nodes are involved).

Critics have countered that the superior results are primarily due to the strict selection criteria used in the adjuvant high-dose chemotherapy trials; these criteria were not used in older trials of adjuvant chemotherapy. Crump and colleagues (164) report that 23% of patients referred for a trial of adjuvant, high-dose chemotherapy were found to have metastatic disease, which made them ineligible. Metastatic disease in these patients would not have been detected with the eligibility criteria required by older trials of adjuvant chemotherapy. By implication, some of the historical controls in the trial by Peters and colleagues (161, 163) may have been found to have metastatic disease if they had been subjected to the same rigorous criteria. In addition, irradiation of the chest wall was required in the high-dose chemotherapy trial after disease on the chest wall recurred in several of the first patients treated. The routine administration of tamoxifen may have further reduced the risk for recurrence in patients receiving high-dose chemotherapy.

For example, a recent report by Ung and colleagues (165) demonstrated the potential danger of using historical controls. Sixty-four patients with extensive nodal involvement received three cycles of standard-dose induction chemotherapy and irradiation of the chest wall and regional nodes, followed by additional standard-dose adjuvant

chemotherapy. The 5-year actuarial freedom from distant relapse rate and overall survival were 45% and 65%, respectively. These results were similar to those reported by Peters and colleagues (161, 163) for patients receiving adjuvant high-dose chemotherapy. Thus autologous bone marrow transplantation is a promising technique for patients with high risk primary breast cancer. New high-dose chemotherapy using taxal and yttrium-labeled monoclonal antibodies directed against breast cancer antigens are also being developed that may be more effective than current therapy when used in conjunction with autologous bone marrow transplants.

2. *Ovarian cancer*

Cancer of the ovary is the sixth most common cancer in women and affects 1-2% of women during their lifetime (166-168). Because of the lack of early symptoms, approximately 75-80% of the patients present with stage III or IV disease (169). Systemic chemotherapy plays a significant role in the treatment of these patients. Approximately 60-80% of the patients with advanced ovarian cancer will have objective responses with platinum-based chemotherapy, and 50% of the patients will achieve clinically complete remission (CR). However, pathologically proven CR is obtained only in 28- 35%, and no more than half of these patients will obtain durable remission (170, 171). Thus, the 5-year actuarial survival of stage III and IV ovarian cancer are 23% and 14%, respectively (169). The contrast of a high response rates with major reduction in tumour burden and low numbers of durable complete remissions indicate the presence or development of resistant subpopulations of tumor cells causing relapse of the disease (172). In a retrospective analysis, both the clinical response and survival rates of patients with ovarian cancer correlated positively with the dose-intensity of the chemotherapy delivered (173-176). Several studies have demonstrated antitumor activity with high-dose cisplatin in patients with ovarian cancer that was refractory to lower doses of the drug. The available data show that the patients with low tumor burden have achieved the best response to high-dose therapy with stem cell transplants (177-181). The initial studies on dose-intensive first-line therapy have given encouraging results. Consequently, the current ongoing trials have focused on testing the potential of this treatment modality in inducing long-term disease-free survival in patients with low tumour burden and chemosensitive disease.

Although the number of patients with stage III and stage IV ovarian cancer who are treated with high-dose chemotherapy with cyclophosphamide cisplatin, and thiotepa and stem cell transplantation is relatively small, the therapeutic results are similar and quite consistent. When the data from these studies are compiled, more than 200 women with advanced stage ovarian cancer have receive high-dose chemotherapy and stem cell transplants for their disease. A major feature of the vast majority of the studies is the strikingly high response rate achieved with intensive therapy. The response rates and long-term disease-free survival rates with standard salvage chemotherapy for advanced ovarian cancer are commonly reported to be less than 20% and 10%, respectively (182). The response rates are in excess of 70% with intensive marrow-supported therapy, usually in heavily chemotherapy pretreated ovarian cancer patients, suggest promise for this technique. If a 75% response rate can be obtained in patients with ovarian cancer refractory to standard therapy, then applying the same treatment earlier in the disease course, when the tumors will be less resistant, may produce more durable antitumor effects. The data of Dauplat et al. showing a 30% progression-free survival for patients with residual disease of 2 cm or less who are treated with high-dose chemotherapy and autologous stem cell transplantation, a group with a very poor prognosis following standard therapy, substantiate this premise (183).

Data on the use of high-dose chemotherapy with stem cell transplantation as the first-line therapy in the treatment of ovarian cancer is limited. However, the first published trials including almost 100 patients have given promising results (178-181). A 4-year survival of 62% and a progression-free survival of 57% survival was seen in a group of 20 patients with stage III-IV patients treated with high-dose therapy supported by stem cell transplantation. Sixteen patients were left with 0.5- 2.0 cm residual disease after primary cytoreductive surgery and the remaining four patients underwent surgery to achieve similar tumour burden before high-dose therapy and high-dose chemotherapy. Within 2 weeks from the primary surgery the patients were treated with two courses of induction chemotherapy including cisplatin and cyclophosphamide followed by high-dose chemotherapy consisting of cisplatin, etoposide, carboplatin and autologous bone marrow transplantation. At second-look laparotomy, pathologically proven complete response was seen in seven (37%), partial response in nine (47%) and no change in three (16%) patients (180). All patients with pathologically proven complete response or only microscopic disease in the lymph nodes prior to high-dose chemotherapy were disease free at the time of the report.

The current data on the use of high-dose chemotherapy with autologous stem cell transplantation in the treatment of advanced ovarian cancer indicate that this approach does not significantly improve survival in patients with refractory or chemo-resistant tumours. On the other hand, good response rates and survival obtained with high-dose therapy in patients with low tumour burden either as first-line treatment or as salvage therapy are very promising. It seems reasonable to use dose-intensive therapy early in the course of the disease before development of resistant cell populations and preferably at a time of minimal tumour burden. Patients with gross or measurable residual tumours after the primary operation should first be treated with a few courses of conventional chemotherapy to evaluate chemosensitivity and high-dose therapy and stem cell transplantation might be given for responding patients only.

H. Approaches To Increase the Efficacy of High-Dose Chemotherapy and Stem Cell Transplantation

Attempts to improve the systemic cytotoxic therapy for preparative regimens used in high-dose chemotherapy and stem cell transplantation by either increasing the intensity of the treatment or by the addition of more agents, have met with limited success (184). Bone marrow transplant preparative regimens are at or near nonhematologic dose-limiting

Potential Complications of Autologous Transplantation

Hematologic toxicity from the preparative regimens
Organ toxicity from the preparative regimens
Post-transplant immunodeficiency
Post-transplant infections
Secondary malignancies

toxicity, making further escalation of the regimens difficult. Furthermore, tumor remaining after high-dose chemotherapy probably represents cells that are very drug resistant. Therefore, immunologic approaches may offer the best chance of increasing tumor cell kill after autologous bone marrow transplantation. They should be non-cross-resistant with cytotoxic agents and possibly could be added without substantially increasing toxicity. An immunologic graft-versus-tumor effect appears to be the major reason for the lower relapse rate after allogeneic bone marrow transplantation compared with that after autologous bone marrow transplantation (185, 186). However, in general, allogeneic bone marrow transplantation has not substantially improved the disease-free survival of patients with lymphomas and acute leukemias compared with autologous bone marrow transplantation. This is because the decreased relapse rate associated with allogeneic bone marrow transplantation is offset by an increased mortality resulting from graft versus host disease.

One immunologic approach being studied for eradicating tumor after autologous bone marrow transplantation is to induce an autoimmune reaction after autologous bone marrow transplantation that resembles allogeneic bone marrow transplantation. A syndrome indistinguishable from mild graft versus host disease can be induced with cyclosporin in rats and mice undergoing syngeneic bone marrow transplantation (187, 188). This syndrome resulted in an immunologic antitumor activity in the animal models (187, 188). A similar syndrome can also be induced with cyclosporin in patients undergoing autologous BMT (189, 190). This autologous graft versus host disease involved only the skin, was self-limited, and therefore did not increase transplant-related toxicity. Preliminary evidence suggests that this syndrome produces clinical immunologic antitumor activity similar to that of allogeneic graft versus host disease but without added toxicity (191).

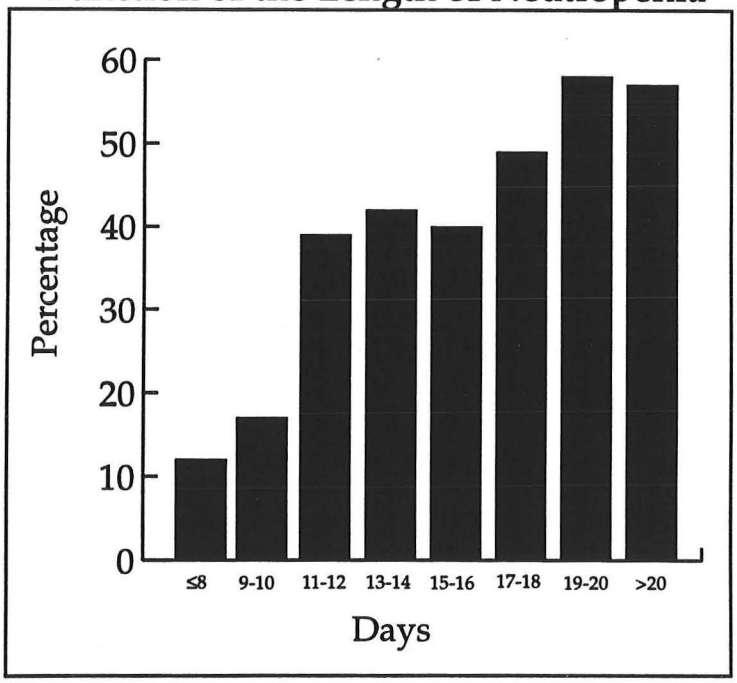
The addition of interleukin-2 after autologous bone marrow transplantation is also being investigated as a means of improving tumor control. Interleukin-2, with or without the addition of activated lymphocytes, shows activity against a wide variety of human malignancies (192, 193). Autologous bone marrow transplantation patients have substantial numbers of circulating lymphocytes with enhanced *in vitro* tumor cell cytolytic activity after incubation with interleukin-2 and anti-CD3 monoclonal antibody (194). Interleukin-2 can be tolerated when given after autologous bone marrow transplantation (195). Interleukin-2 was administered for 6 to 12 days was administered to 19 patients with refractory solid tumors beginning one day after bone marrow transplantation. The toxicity was similar to that seen in studies with interleukin-2 given in the non-bone marrow transplantation setting. Studies will be needed to determine if this approach will improve the disease-free survival after autologous or stem cell transplants.

Finally, complications from autologous transplantation in conjunction with high-dose chemotherapy must be decreased. Infectious complications occur in about 10% of patients undergoing high-dose chemotherapy and autologous transplantation with a overall mortality of 1-3% (196). The infectious complications were markedly increased in the face of prolonged neutropenia. Thus by increasing the number of stem cells infused in conjunction with treatment using multiple hematopoietic growth factors, the period of neutropenia after high-dose chemotherapy may be decreased resulting in fewer infectious complications.

Infectious Complications of AutoBMT

Number of Patients	144
Any infection or isolated fever	25.7%
Isolated fever	18.1%
Septicemias	3.5%
Pneumonia	2.1%
Skin infections	0.7%
Other infections	2.1%
Invasive fungal infections	0.7%
Death	2.8%

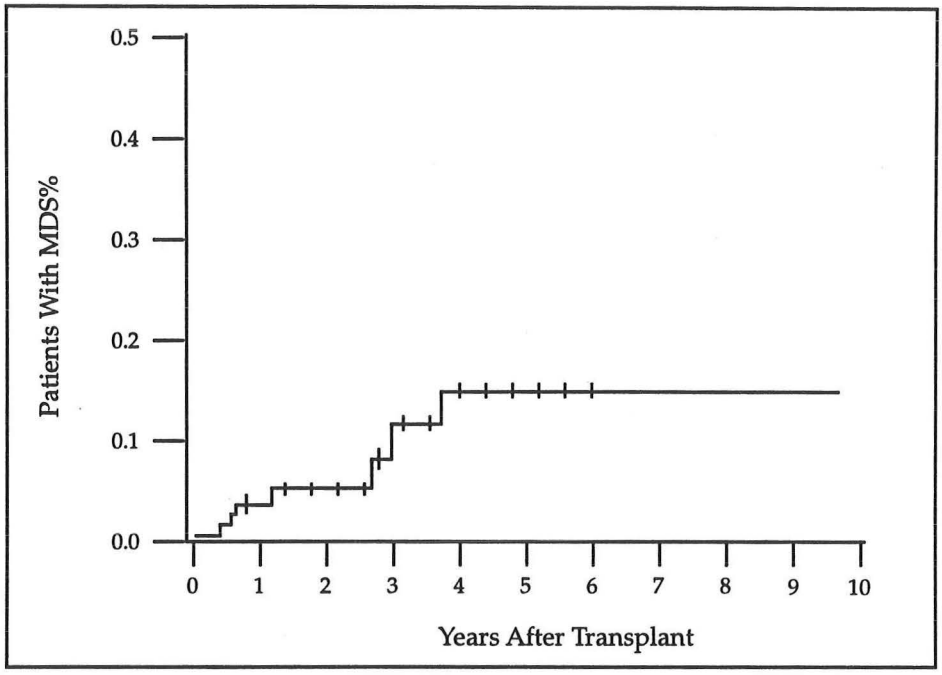
Infectious Complications of AutoBMT as a Function of the Length of Neutropenia



Another potentially serious complication of high-dose chemotherapy and autologous bone marrow transplantation is myelodysplasia (197, 198). In two studies of patients who were treated with high-dose chemotherapy and autologous transplantation for non-Hodgkin's lymphoma, the actuarial risk of acute myeloid leukemia or myelodysplasia was between 15-18% with a median onset of 30 months post-transplant. Factors that correlated with development of myelodysplasia were an increased duration of exposure to conventional chemotherapy especially alkylating agents and concurrent radiation therapy

prior to autologous transplantation. These results indicated that extensive conventional chemotherapy treatment should be limited if high-dose chemotherapy regimens are planned.

Incidence of Myelodyplastic Syndrome After ABMT



I. Future Trends in Autologous and Stem Cell Transplantation

The indications for stem cell transplantation is growing and surpasses that of autologous bone marrow transplantation. The short time regimen for post-transplant hematopoietic recovery, the lower cost of the procedure, and the availability of stem cells even in the case of metastatic involvement or of hypocellularity of bone marrow favor the use of this procedure. At present, non-Hodgkin's lymphomas and specifically low-grade NHL, refractory or relapsing Hodgkin's disease, and intermediate or high-grade multiple myeloma emerge as the more promising candidates for high-dose chemotherapy and stem cell transplantation in the future. There is also promise in patients with breast cancer having more than 10 positive axillary nodes and in ovarian cancer with minimal residual disease.

Mobilization regimens and collection monitoring of BSC need to be improved. The role of newer cytokines in such a procedure should be better defined. Using these cytokines alone might be a new field of development for BSC mobilization. The aim of an adequate BSC collection with a single leukapheresis is attainable (199). Additionally, the risks due to aplasia following chemotherapy would be avoided. The use of ex vivo cytokine-expanded blood-derived hematopoietic progenitors might better accelerate hematopoietic recovery (200). For example, patients who had stem cells mobilized with high-dose chemotherapy and G-CSF treatment underwent a single apheresis (200). CD34 positive cells were selected and grown for 12 days in culture in the presence of IL-1, IL-3, IL-6, erythropoietin, and stem cell factor. CD34 positive cells were expanded by more than 60-fold during this *in vivo* expansion procedure and when these cells were infused

into patients they were able to reconstitute hematopoiesis as well as *in vivo* isolated stem cells (200).

Ex vivo Expansion of Stem Cells

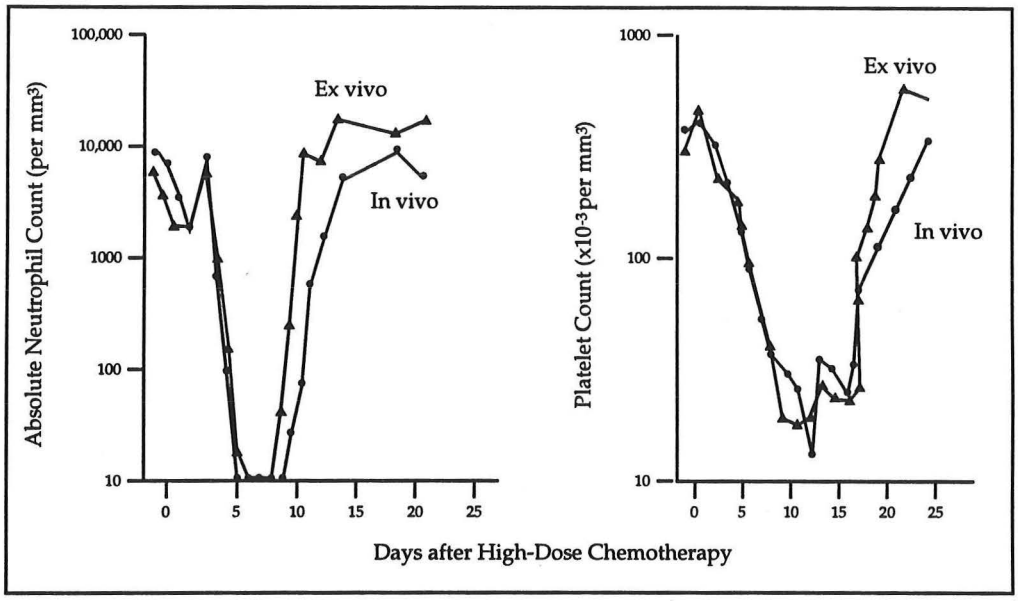
Adequate collection from 100-200 ml of blood.

Grown in culture with stem cell factor, IL-1, IL-3, IL-6 and erythropoietin resulting in 60-fold expansion.

Small amount of blood needed reduces tumor contamination by 4 orders of magnitude.

Effective in reconstituting myeloid and platelet counts after high dose chemotherapy.

Hematopoietic Reconstitution Using Ex vivo Derived Stem Cells



This procedure is attractive because of the limited amount of apheresis necessary (100-200 ml of blood) and the marked reduction in tumor cell contamination with the small blood samples needed for this procedure. The *in vivo* use of cytokines might also hasten significantly hematopoietic recovery following transplantation of blood-derived CD34 positive cells purified either by a biotin-avidin monoclonal antibody system (201) or a immunomagnetic procedure (202).

Fewer relapses are observed after allotransplants as compared with autologous procedures, which is closely related to the graft-versus-host/ graft-versus-leukemia effect. Adjuvant immunotherapy, using for example post-transplant continuous infusion of low

doses of cyclosporin A (189, 190) or IL-2 (192) might induce the graft-versus-tumor effect which is normally absent following autotransplants. This could consequently reduce the incidence of post-transplant tumor recurrence.

Stem cell transplants have proven to be safer, cheaper and more comfortable for patients than autologous bone marrow transplants. The development of stem cell transplants has contributed to the enhanced study of multipotent hematopoietic stem cells. Given their immense potential of this procedure, it is unclear whether stem cell transplants and high-dose chemotherapy can significantly alter the overall survival of the various diseases in which they are performed remains to be clearly demonstrated. It is now the major question that must be definitively be answered.

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