# Medical Progress

## Bone Marrow Transplantation
### Part II—Autologous

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*Autologous bone marrow transplantation provides an effective form of "rescue" following high-dose therapy used for treating certain malignant diseases. The high doses of radiotherapy or chemotherapy, or both, should allow for greater tumor cell kill if dose-response to therapy exists for that tumor. The use of autologous bone marrow obviates the need for an HLA-identical donor, and the need for pretransplant immunosuppression; no graft-versus-host disease would ensue. We review in part II the history and background, methods of obtaining autologous stem cells, and details of the results achievable with this type of therapy. We discuss potential difficulties with autologous transplantation, as well as possible future areas of research.*

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Autologous bone marrow transplantation developed from the success of allogeneic transplants. The concept, however, dates to the 1950s, when the first clinical study was published.[1] Over the ensuing several years, more than 50 such cases were reported.[2-8] These early trial results were disappointing, as they did not show improved antitumor effectiveness or that autologous bone marrow was even necessary to shorten the time of aplasia. With the encouraging results and true cures using allogeneic transplantation reported by Thomas and colleagues, interest in autologous transplantation was rekindled.[9]

Using autologous marrow obviates the need for an HLA-identical family donor (the chance of an HLA-identical donor-recipient pair being found among siblings is one in four). Autologous bone marrow transplantation also removes the need for pretransplant immunosuppression so that antitumor therapy can be based on the optimal combination of active agents. Finally, there is no graft-versus-host disease, a major source of direct or indirect morbidity and mortality. Because of these factors, autologous transplants can be offered to a larger and older group of patients.

There are several caveats. The most obvious is that when an autologous transplant is considered, the marrow should be free of the tumor cells, or at least small numbers of viable tumor cells (not detected in the bone marrow) should not substantially affect disease-free survival or cures. The tumor being treated should exhibit a steep dose-response curve to the therapeutic agent so that a higher dose will substantially increase cell kill. If one envisions cure as follows:

$$\text{cure} \propto \frac{\text{active agent(s)} \times \text{dose}}{\text{tumor burden}}$$

bone marrow transplantation allows an investigator to manipulate the dose and the active agent(s). The maximum tolerated dose with or without bone marrow transplantation may be only severalfold higher (Table 1), but an important concept is that for many experimental malignant neoplasms, a twofold increase in drug concentration may result in a tenfold greater cell kill. Also, the toxic effects from the regimen should be ablative to the marrow so the reinfusion of bone marrow becomes a necessary aspect of the therapy.

Experimental studies in animals showed conclusively the protective effect from total body radiotherapy by reinfusion of autologous marrow.[18,19] Several early studies suggested the same protective effect in humans.[3,20-24] Current excitement for autologous transplantation came from studies by the National Cancer Institute in patients with lymphoma, where Appelbaum and colleagues showed that relapsed patients with Burkitt's lymphoma could be cured by high-dose therapy.[21] Autologous bone marrow transplantation shortened the period of aplasia. From these studies of lymphoma, concepts particular to autologous transplantation have evolved, and, currently, notable efforts are directed to using autologous bone marrow transplantation in other malignant disorders.[25] To date, however, there have been no prospective randomized studies showing a benefit in favor of bone marrow transplantation. Such studies are ongoing.

### Technical Aspects

The technical aspects of bone marrow harvesting have been described previously. There are, however, several steps that are unique to autologous bone marrow transplantation. First and foremost, the harvested bone marrow usually needs to be stored. Because the recipient and the donor are one and the same and the recipient needs myeloablative therapy, the marrow is stored until a later date to allow for the therapy to be administered and for any drug to be metabolized and excreted. The time period between harvesting and reinfusion of marrow may vary from several hours to months. Occasionally the period from harvest of marrow while the patient is in remission to its use at the time of relapse is several years.

Marrow viability may be maintained by proper cryopreservation and storage in ultralow temperatures. The tempera-

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TABLE 1.—*Dose Escalation Studies With Autologous Bone Marrow Transplantation*

| Agent (units) | Dose | | | Limiting Toxicity |
|---|---|---|---|---|
| | | Autologous Bone Marrow Transplant | | |
| | Conventional | Maximum Without | Maximum With | |
| Total body irradiation (rads)[10,11] | 150 | 350 | 1,000* | Lung |
| Mechlorethamine HCl (mg/kg)[12] | 0.4 | 1.6 | 2.5 | Central nervous system |
| Cyclophosphamide (mg/kg)[13,14] | 50 | 200 | 200 | Heart |
| Carmustine (mg/m²)[15] | 200 | 600 | 1,200 | Liver, lung |
| Melphalan (mg/m²)[16] | 35 | 140 | 180-200 | Liver, gastrointestinal tract† |
| Etoposide (mg/m²)[17] | 360 | 1,200 | 2,400 | Gastrointestinal tract† |

*Maximum of 1,400 rads in split fractions.
†Includes stomatitis.

ture of storage may range from −80° to −196°C (liquid nitrogen). A crucial step is the cryopreservation. The pluripotent stem cell must be protected from damage caused by the freezing. Cryoprotectants are agents such as glycerol or dimethyl sulfoxide.[26] Dimethyl sulfoxide is the most widely used agent today. Although the protective effect of these agents is still incompletely understood, using these agents and controlled rate freezing, minimizing the duration of the plateau of heat transition, has resulted in effective storage. Controlled cooling is thought to be very important, as the rate must be slow enough to prevent intracellular ice formation but rapid enough to prevent extracellular ice formation. This is usually achieved by cooling at 1°C to 2°C per minute using a programmable cooling chamber. There is a negligible loss of viability judged by the restoration of hematopoiesis.

Before cryopreservation, red blood cells and granulocytes should be removed because these cells are not effectively preserved and may cause clumping of the marrow. This can be achieved by various methods such as dextran sedimentation, differential centrifugation, or Ficoll-Hypaque centrifugation.

Also unique to autologous bone marrow transplantation is the attempt to remove obvious or possible contaminating tumor cells from the marrow. This process is known as purging. Various methods have been tried, all aimed at exploiting specific differences between tumor cells and normal hematopoietic precursors. Clearly the purging process must be potent enough to remove several logs of tumor cells, yet gentle or specific enough to spare the hematopoietic stem cells. Purging in experimental animal models shows a clear effect of this process on survival.[27-31]

Results of several studies have suggested that purging of the marrow is effective. Gorin, in a review of the European Bone Marrow Transplant Registry results, noted a difference in patients receiving autologous bone marrow transplant for acute nonlymphoblastic leukemia.[32] Patients' bone marrows were purged with mafosfamide. The beneficial effect mainly was significant in the group of patients who were transplanted early—less than six months after achieving a complete remission. This study was retrospective, however, using registry data of many centers that used a variety of preparatory regimens. Other investigators using either 4-hydroperoxycyclophosphamide or monoclonal antibodies without control groups have reported improved survival rates in transplanted patients.[33,34] Because of the high recurrence rate in diseases where autologous transplantation has been used, the contribution of purging may be difficult to establish.

Another unique aspect to autologous transplantation is the use of peripheral stem cells. Animal studies using dogs have shown the protective effect of peripheral blood mononuclear cells against lethal myelotoxicity.[35] In 1979 Goldman and co-workers reported successful autologous peripheral stem transplantation in patients with chronic myelogenous leukemia.[36] Failures were also reported at that time. Encouraged primarily by the animal experiments, the University of Nebraska group and several other centers continue similar efforts in humans.[37] Unfortunately, there is no good assay for the true stem cell except for in vivo proof of engraftment. Assays such as granulocyte macrophage colony-forming units measure hematopoietic precursors and not the activity of the true stem cell. This assay and the number of mononuclear cells are useful as relative indicators of the number of stem cells. The in vivo data are becoming available and clearly support the experience from the laboratory. Peripheral stem cells, especially when collected at the time a patient is recovering from standard chemotherapy, are able to fully reconstitute the marrow of patients who have had myeloablative therapy. In fact, the time to engraftment from these peripheral cells is shorter than when using marrow. The average time to 500 granulocytes per microliter is approximately two weeks.

The idea of autologous peripheral stem cell transplant has several attractive features. Cells can be collected by repeated apheresis, and there is no need for marrow harvesting and possible general anesthesia. This is especially useful for some patients who have no harvestable marrow, such as patients with Hodgkin's disease or lymphoma after pelvic radiotherapy. Autologous peripheral stem cell transplants may also allow transplantation for patients with possible marrow disease, such as those with Hodgkin's disease with fibrosis of the marrow and possible marrow involvement. Whether clonogenic tumor cells circulate freely in the peripheral blood and are also collected is unknown at this time. In cases of failure from transplantation, the sites of relapse are usually in areas of previous disease, suggesting that persistent disease and not reinfusion of tumor cells accounts for the recurrence.[37] The malignant cells in circulation may not have a high fraction of clonogenic cells and thus may be less able to establish recurrent disease. Furthermore, freezing and thawing may contribute to more selective removal of the tumor cells when or if they are present in small numbers. There are reports of apparent tumor cells being found in normal marrow collected in patients for breast cancer and lymphoma when the marrow was grown in long-term cultures.[38] Whether these represent especially virulent cells or possibly artifacts of long-term cultures remains to be determined.

## Results

What, then, are the results with autologous bone marrow transplantation? The answer to this question will depend on how autologous transplantation is used. One can envision using autologous transplantation for various reasons:

- To deliver curative amounts of active agent(s) that would otherwise not be possible because of marrow toxicity;
- To determine the nonmyeloid dose-limiting toxicity of single agents and antitumor activity; or
- As "rescue" to prevent cumulative toxicity to bone marrow because of prolonged exposure to conventional chemotherapy.

Beginning with the third point, using autologous marrow as "rescue" for conventional therapy has been tried. The cell dose used was not specified but seemingly resulted in only minor delays in therapy and, thus, good dose intensity.[39] Whether this is an important effect applicable to a variety of patients remains to be determined.

Using autologous bone marrow transplantation to determine the nonmyeloid dose-limiting toxicity of single agents and potential antitumor activity has been a crucial step in developing a rational therapeutic protocol. The use of single agents allows the determination of the nonmyeloid dose-limiting toxicity (Table 1). These single agents, usually used in phase I or II trials, allow the evaluation of antitumor activity such as of carmustine (BCNU) therapy for central nervous system tumors and melphalan for neuroblastoma and melanomas.[15,16] As shown in Table 1, the increment of each single agent achievable with autologous bone marrow transplantation is modest, and such increments are unlikely to achieve a notable effect on cure rates when used alone in resistant tumors. With this knowledge, however, an investigator can then combine drugs rationally, exploiting the optimal combination of active agents with different limiting organ toxicity. These toxicity data are not available from conventional doses of the listed agents. Note, however, that the "maximum" dose without autologous bone marrow transplantation allows the severe pancytopenias to occur, lasting no more than three to four weeks. Thus, it is unlikely that combinations of the maximum doses of the drugs with or without radiation would consistently allow for recovery without bone marrow transplantation.

The results of autologous transplants used to deliver curative concentrations of active agents are the most promising in clinical trials. Most of the diseases will be discussed individually.

### *Lymphomas*

*Diffuse large cell lymphoma.* In the 1970s Appelbaum and colleagues did bone marrow transplantations in eight patients with non-Hodgkin's lymphoma, using the standard preparative regimen consisting of total body radiotherapy and cyclophosphamide. In one study all patients had advanced refractory disease and had failed earlier therapy.[40] All but one achieved a complete response, and three of the eight are still alive and in complete remission. These data gave impetus to several other trials in bone marrow transplantation for lymphoma using allogeneic and autologous bone marrow with or without irradiation. Autologous bone marrow transplantation was used with Burkitt's lymphoma patients, resulting in more rapid recovery of peripheral blood counts.[21,41] The cumulative experience from the literature still only represents several hundred patients, although the number continues to grow exponentially. From these early studies, several concepts have evolved. The major one is that of tumor responsiveness. Various groups have shown that a so-called sensitive relapse patient—that is, a relapsed patient who is still responding to conventional therapy—does significantly better than a patient who is in resistant relapse—refractory to second-line therapy. Similarly, the lower the tumor burden before ablative therapy, the more likely it is that the outcome will be favorable.

Recently reported results have been encouraging. Several groups from the Dana Farber Cancer Center (Boston), Memorial Sloan Kettering (New York), Lyon (France), the University of Nebraska Medical Center (Omaha), and MD Anderson Cancer Center (Houston) have reported excellent survival rates and probable cures in patients with relapsed lymphomas (predominantly diffuse large cell) ranging from 65% to 70%, with follow-up periods of two to four years.[34,42,43] Furthermore, transplantation has been used in this group of patients in an "adjuvant" setting. That is, retrospective analysis is used to identify risk factors for relapse. These factors are then used if a patient currently on therapy has a high likelihood of relapse. In such patients, once a maximum response or a complete response has been achieved, transplantation can be used with the goal of helping the patient achieve a cure. In the study by Gulati and colleagues from Memorial Sloan Kettering, autologous transplantation did accomplish this, taking a group of patients with historically about a 20% to 30% chance of cure and improving the actuarial disease-free survival to approximately 80%.[41] One caveat is that patients in several of the studies were carefully selected, and part of the inclusion criteria was a responsive relapse or the achievement of a "minimal disease state." Thus, other patients who did not achieve a minimal disease state were not eligible. If these patients are included in the calculation, the data may approach results reported using second-line conventional chemotherapy for relapsed patients. This selection bias should not, however, detract from the excellent results achieved for this specific high-risk group of patients. Only a prospective randomized trial comparing transplantation with continued conventional chemotherapy will determine the best therapeutic option.

*Hodgkin's disease.* Autologous bone marrow transplantation for Hodgkin's disease is now a highly successful treatment for patients who have failed first-line chemotherapy. Before autologous transplants were available, the optimal management for patients failing the primary use of mechlorethamine hydrochloride, Oncovin, procarbazine hydrochloride, and prednisone (MOPP) therapy or for those who did not achieve a complete response, was to use a second-line, non-cross-resistant regimen, usually doxorubicin (Adriamycin) hydrochloride, bleomycin sulfate, vinblastine sulfate, and dacarbazine. In the Stanford experience, however, the freedom from progression was only 20% in complete responders and only 9% if all patients were analyzed. Encouraging results have been obtained for these patients with autologous transplantation, including those patients who fail second-line or further chemotherapy and clearly represent a poor prognostic group. Various investigators have done autologous transplants for such patients and found a disease-free survival rate of 25% to 70%.[44–46] When the MD Anderson and University of Nebraska group evaluated their

data, they were able to subdivide the patients into high-risk and low-risk groups. Those patients with good performance status, low tumor burden, and having previous therapy with two or less drug combination regimens had a freedom from progression of approximately 80% at four years; most of these patients are probably cured.[47] Again, a word of caution: comparing autologous bone marrow transplantation data with chemotherapy is necessary because there is usually an age limit with transplantation. Further carefully designed studies with larger numbers of patients are necessary to confirm these results. It is still unclear whether the concept of tumor responsiveness applies to this group of patients.[46] For Hodgkin's patients who have had previous mantle radiotherapy, a preparative regimen should not contain total body irradiation, since further radiotherapy leads to substantially increased pulmonary toxicity.

*High-grade lymphomas.* Burkitt's lymphoma is historically important because it was one of the first tumors clearly curable with chemotherapy. Despite its sensitivity to chemotherapy, survival rates are dismal in patients with advanced or relapsed disease. Encouraged by the results of allogeneic transplants for leukemias and the similar high growth fraction of Burkitt's lymphoma, investigators attempted autologous transplantation. A remarkably high response rate was noted when the dose of chemotherapy was increased with autologous transplants compared with the resistance observed at conventional doses. Long-term survival and probable cure in these early patients left no doubt about the efficacy of this treatment. Although the total number of patients receiving the procedure is relatively small, the overall survival rate in those with poor prognostic factors is approximately 50% to 70%.[21,41,48] Autologous bone marrow transplants for high-risk lymphoblastic lymphoma also have had encouraging results.[49,50]

*Other lymphomas.* Autologous bone marrow transplantation to treat other lymphomas such as follicular small cleaved cell and diffuse mixed cell have also been done. Likewise, patients with low-grade lymphomas that transform to a higher grade lymphoma have also received transplants. Some of the results are encouraging, but the number of patients is still too small for any meaningful conclusions.

### *Leukemias*

Intensive ablative regimens have also been tried in autologous transplantation for acute leukemias such as those successfully treated with allogeneic bone marrow transplantation. Leukemia, as a disease of the bone marrow, makes autologous transplantation difficult. Assuming no major immunologic changes in the bone marrow by the processing method, one would not expect the results of autologous transplants at best to be very different from identical twin transplantation, which carries a high relapse rate of approximately 50% to 60%.[51] The high relapse rate reflects an inadequate preparative regimen and the lack of a graft-versus-leukemia effect. Newer preparative regimens should lower the relapse rate. Clearly, the primary concern of autologous bone marrow transplantation for leukemia is reinfusion of clonogenic cells. The assumption is that when marrow is harvested from a patient in complete remission, it is contaminated with a small number of leukemic cells. The mechanical handling and the freezing and thawing may eliminate a good fraction of these cells, and small numbers reinfused may not necessarily lead to leukemia relapse. Several pilot studies have been done with encouraging results.[52-57] When autologous transplantation is done for acute nonlymphoblastic leukemia in first complete remission, the overall results show an approximately 35% to 50% chance of disease-free survival. These results may be favorably biased by the timing of the transplant, such as doing the transplant several months after the patient has remained in complete remission, or by other selection factors. Prospective studies are needed to confirm the early encouraging results comparing allogeneic bone marrow transplantation with continued intensive chemotherapy. Investigators at Johns Hopkins University (Baltimore) have reported promising results with autologous transplantation for treating acute nonlymphoblastic leukemia patients in second or subsequent complete remissions.[33] In these studies using 4-hydroperoxycyclophosphamide purging, a 28% disease-free survival rate at four to five years has been reported—a good outcome in a group of patients with a very poor prognosis.

Autologous transplantation for acute lymphoblastic leukemia has also been tried. The results so far have been discouraging and do not seem to be significantly different from those attained with intensive chemotherapy.[58]

### *Solid Tumors*

A clear advantage to using autologous transplantation is dose intensity. Several studies have suggested a steep dose-response curve for irradiation and chemotherapy in patients with breast cancer, making this disease a likely candidate for autologous bone marrow transplantation. A recent review of the available data has been published by Antman and Gale.[59] It is clear that in unfavorable groups of patients, the results are encouraging, but it is still quite early. The important point is that using intensive therapy does achieve higher complete response rates.

In 1988 Peters and associates reported early data on using autologous transplantation in ten breast cancer patients with a highly unfavorable prognosis.[60] These were premenopausal women with more than ten positive lymph nodes. In such patients, there is usually a median time to relapse of a year and a survival of only three to four years. They were initially treated with cyclophosphamide, doxorubicin, and fluorouracil (CAF). Bone marrow was then harvested and one more cycle of CAF given. Following this, patients were prepared with high doses of cyclophosphamide, cisplatin, and carmustine. They then received their stored bone marrow. All ten patients survived the transplant, and none have relapsed; however, the median follow-up time is still less than a year. This promising result indicates that this therapy may have a notable effect in curing such patients.

A second type of tumor where autologous bone marrow transplants have been shown to be effective is neuroblastoma, one of the most common childhood malignant tumors. In children older than 1 year with neuroblastoma, 70% have stage IV disease, which is fatal in 90%. Initial studies using high-dose melphalan and autologous bone marrow transplantation showed some promise.[61-63] Stimulated by these early results, several groups used autologous transplantation following aggressive surgical procedures and chemotherapy. Pinkerton and co-workers carried out a randomized study with the European Neuroblastoma Study Group and reported a significant improvement in disease-free survival rates in those patients who received high doses of melphalan and autologous bone marrow transplantation.[64] With follow-up

of as long as 48 months, the disease-free survival plateau for transplanted patients is at approximately 40%, compared with 20% for those patients not receiving transplants.

Several other tumors have also shown some response to transplantation. These include sarcomas,[65] melanomas,[66] small-cell lung cancer,[67,68] colon cancer,[69] and multiple myeloma.[70,71] These trials have been done with small numbers of patients and have not shown a significant effect on disease-free survival rates yet.

## Future Directions

Clearly the earlier use of autologous transplantation as adjuvant therapy will have a greater effect and likely more meaningful results. Autologous transplants earlier in the clinical course of the underlying diseases have already been done with hematolymphoid disorders and should also affect response rates in patients with solid tumors. Well-designed, prospective, randomized studies are still lacking, however.

New combinations of active agents, including drugs and cytokines such as interferon or interleukin 2, used either as part of the preparative regimen or potentially as immunomodulators, may affect the outcome of autologous transplants. Other immune system manipulation such as adoptive transfer with lymphocyte-activated killer cells or tumor-infiltrating lymphocytes may also contribute to a more favorable outcome. Recently, Santos and colleagues reported the induction of what appears to be graft-versus-host disease in patients with autologous transplants who are taking cyclosporine. If this graft-versus-host disease is limited to a mild clinical manifestation and associated with a graft-versus-tumor effect, it could contribute to a lower relapse rate.[72]

The reported isolation of the mouse hematopoietic stem cell by Weissman's group[73] has been very exciting, and the hope is justified that the human stem cell will also soon be isolated. The availability of the human stem cell would be an important step forward for autologous bone marrow transplantation, lessening the concern of reinfusion of tumor cells, as presumably stem cell markers will not be found in tumor cells.[74]

The use of cloned growth factors may reduce morbidity and mortality from prolonged neutropenia but may also aid selection of a subgroup of patients for transplantation. That is, current dose intensity may be achievable without transplantation. Potentially, a larger number of patients may be curable without transplants.

Finally, as investigators understand better the toxicities and indications for autologous bone marrow transplantation, the patients' burden in terms of physical, psychological, and financial costs should lessen substantially.

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