Allogeneic Hematopoietic Transplantation for Acute and Chronic Myeloid Leukemia: Non-myeloablative Preparative Regimens and Induction of the Graft-versus-leukemia Effect

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Current Oncology Reports 2000, **2**:132–139
Current Science Inc. ISSN 1523–3790

High-dose chemoradiotherapy with allogeneic blood or bone marrow transplantation is an effective and potentially curative treatment for advanced or high-risk hematologic malignancies, but it has been associated with significant morbidity and mortality resulting from toxicity of the preparative regimen, graft-versus-host disease, and the immunodeficient state that accompanies the procedure. Development of safer and less toxic treatment has been the subject of much research. This review summarizes the current understanding of the mechanisms by which allogeneic transplants cure leukemia and the rationale for non-myeloablative preparative regimens. Experience of the authors is related with 116 patients diagnosed with acute or chronic myeloid leukemia who underwent allogeneic hematopoetic transplantation with two non-ablative regimens that differed in intensity.

Introduction

High-dose chemoradiotherapy with allogeneic blood or bone marrow transplantation (BMT) is an effective, potentially curative treatment of advanced or high-risk hematologic malignancies [1–3]. However, it is associated with significant morbidity and mortality due to the toxicity of the preparative regimen, graft-versus-host disease (GVHD), and the immunodeficient state that accompanies the procedure. Much of the benefit of allogeneic BMT is mediated by an immune graft-versus-malignancy effect. Extensive research has been directed toward the development of safer and less toxic approaches to allogeneic transplantation. This would also permit wider application of a potentially curative treatment to elderly patients or those with organ dysfunction precluding high-dose therapy. In this review, we discuss the current understanding of the mechanisms by which allogeneic transplants cure leukemia and the rationale for transplants using non-myeloablative conditioning regimens. We also report our experience with 116 patients with acute or chronic myeloid leukemia having allogeneic hematopoietic transplantation using two non-ablative regimens that differed in intensity.

How Does Allogeneic Hematopoietic Transplantation Cure Leukemia?

Bone marrow transplantation was initially developed as a means to deliver supra-lethal doses of chemotherapy and radiation for the treatment of the malignancy [1–3]. Myelosuppression is the limiting toxicity of many chemotherapeutic agents and radiation. Hematopoietic transplantation can restore hematopoiesis after high-dose treatment and by doing so can allow escalation of myelotoxic chemotherapy and whole-body radiation doses up to three- to fivefold above the conventional maximal tolerated doses. Many malignancies exhibit a steep dose-response relation to chemotherapy, and increasing the doses may markedly enhance cytoreduction. BMT was initially viewed as a supportive care modality to restore hematopoiesis. It has subsequently become apparent that high-dose chemotherapy does not eradicate the leukemia in many patients and that the therapeutic benefit of BMT relates in part to an associated immune-mediated graft-versus-leukemia effect (GVL) [4,5]. Extensive clinical and experimental data support the presence of a GVL effect. GVL was documented in animal models [6,7] as well as in human clinical transplantation. A higher risk of relapse occurs after T-cell–depleted [8,9] or syngeneic

[10,11] transplants. Patients with acute and/or chronic GVHD have reduced risk of relapse, suggesting a relationship between GVL and GVHD [12–14]. Elimination of residual disease, as detected by cytogenetics or polymerase chain reaction (PCR) techniques in more indolent malignancies such as chronic myelogenous leukemia (CML), may take 6 to 12 months after transplant presumably due to an ongoing GVL effect [15]. Withdrawal of immunosuppression given for prevention of GVHD can occasionally lead to restoration of remission in patients relapsing after transplant [16]. The most direct evidence of GVL is the observation that infusion of donor lymphocytes (DLI) can re-induce remission in patients who relapse after allogeneic transplantation [17•,18•]. This has been most effective against CML. Up to 80% of patients with CML who relapse into a chronic phase achieve complete cytogenetic remission after DLI, with the best results reported in early cytogenetic relapse. Acute myelogenous leukemia (AML) and myelodysplasia are also subject to GVL; about one third of these patients respond to DLI, but remissions are generally transient. Graft-versus-malignancy effects have also been shown in multiple myeloma [19,20], chronic lymphocytic leukemia [21], low-grade lymphoma [22], and solid tumors [23–25].

There are a number of potential antigenic targets for the GVL effect. GVL may reflect immune reactivity against broadly expressed major and minor histocompatibility antigens similar to targets of GVHD. Many patients will achieve an antileukemic response to DLI without developing GVHD, suggesting that minor hematopoietic antigens restricted to hematopoietic tissues, malignancy-specific antigens, or antigens that are overexpressed or abnormally expressed on the malignant cells [5,26,27] may be involved. Intensive research is directed at developing methods for separation of GVL and GVHD and/or generation of a specific tumor-directed immunotherapy.

Hematopoietic Transplantation with Non-myeloablative Conditioning

Due to the increased risk of regimen-related toxicity and GVHD that occurs with advanced age, the use of standard myeloablative preparative regimens with allogeneic transplantation has been generally limited to younger patients who are in good general medical condition. Improvements in supportive care, infection control, and GVHD prophylaxis and treatment have enabled many centers to treat older patients, but only a few centers consider patients older than 55 to 60 years to be eligible for transplantation [28•,29–32]. Leukemia and other malignancies are more common with advanced age. Elderly patients with acute myelogenous leukemia are more likely to have poor prognostic features such as adverse cytogenetic abnormalities and antecedent hematologic disorder and to have a poor prognosis with available therapy [33–35]. Novel therapeutic options need to be explored.

Discovery of the curative potential of the immune-mediated GVL effect has led to a novel therapeutic approach. Low-dose, relatively nontoxic conditioning regimens have been designed, not to eradicate the malignancy but rather to provide sufficient immunosuppression to achieve engraftment and to allow induction of GVL effect as the primary treatment [36•]. The reduced toxicity of the preparative regimen is expected to allow treatment of older patients and of younger patients with comorbidities that preclude the standard ablative preparatory regimen. These regimens have been referred to as "non-myeloablative" if they do not completely eradicate host hematopoiesis and immunity and allow the development of mixed hematopoietic chimerism upon engraftment.

Graft-versus-host disease is one of the major causes of posttransplant morbidity and mortality. Acute GVHD results at least partially from tissue injury and cytokine release secondary to the toxicity of the preparative regimen, which amplifies the immune graft-versus-host reaction [37,38]. Use of a less toxic preparative regimen should theoretically limit this tissue injury and cytokine release and reduce the incidence and severity of GVHD. This is especially important in elderly or debilitated patients who are less likely to tolerate the morbidity associated with GVHD or its treatment.

Similarly, posttransplant immune deficiency results from ablation of host immunity by the preparative regimen. A non-ablative regimen will allow for at least temporary partial persistence of immunity that might provide limited protection from infections. The initial non-ablative treatment is expected to produce only transient suppression of the leukemia, but it also allows time for the immune graft-versus-leukemia effect to develop. Patients with detectable or recurrent malignancy after the allogeneic transplant may respond to additional immunotherapeutic approaches including tapering of immunosuppressive therapy, DLI, or a second non-myeloablative treatment with infusion of additional donor lymphoid cells for disease control. The initial treatment and achievement of engraftment may serve as a platform for additional allogeneic cell-based immune therapies.

Purine Analogue/Cytarabine–containing Non-ablative Regimens

Pilot studies by our group [36•] and by others [39•] have shown that less toxic purine analogue–based non-myeloablative conditioning regimens can provide sufficient immunosuppression to allow engraftment of allogeneic progenitor cells. The recently developed purine analogues, fludarabine and cladribine, exert potent immunosuppressive effects in addition to their antitumor activity against a range of hematologic malignancies [40]. We used two regimens, which are outlined in Table 1. Patients without prior fludarabine exposure received fludarabine, cytarabine, and idarubicin [41]. Patients with prior fludarabine exposure received cladribine

Table 1. Preparative Regimens

	Day pretransplant							
	-7	-6	-5	-4	-3	-2	-1	0
Purine analogue/cytarabine								
Fludarabine (mg/m^2)		30	30	30	30			T
Cytarabine (gr/m^2)		2	2	2	2			R
Idarubicin (mg/m^2)		12	12	12				A
								N
Cladribine (mg/m^2), ci	12	12	12	12	12			S
Cytarabine (gr/m^2)	1	1	1	1	1			P
								L
Purine analogue/melphalan								A
Fludarabine (mg/m^2)		25	25	25	25	25		N
Melphalan (mg/m^2)					90	90		T
Cladribine (mg/m^2), ci		12	12	12	12	12		
Melphalan (mg/m^2)					90	90		

ci—continuous infusion.

Table 2. Patient Characteristics

	Purine analogue/cytarabine regimens	Purine analogue/melphalan regimens
Patients, *n*	46	70
Median age	60 (29–75)	54 (24–70)
>50 years	38 (84%)	44 (63%)
>60 years	26 (58%)	18 (26%)
Sex (Male/female)	21/25	43/27
Diagnosis		
AML/MDS	35	43
CML	11	27
Disease stage at BMT		
Low risk*	24	12
High risk†	22	58
Cytogenetics (percentage diploid)‡	27%	48%
Time to BMT (months)	15 (4–114)	15 (1–220)
Number of prior treatments	2 (1–3)	2 (1–8)
Prior BMT	None	17
Comorbidity (percentage of patients)§	60	47
Donor		
Matched related	35	38
Mismatched related	9	5
Matched unrelated	2	32
Preparative regimen		
Fludarabine-based	34	64
Cladribine-based	12	6

*Disease in remission or chemosenesitive disease, no peripheral blasts, and under 10% bone marrow blasts.
†Disease in relapse.
‡Excluding patients with CML.
§Concurrent medical problem considered a relative contraindication to allogeneic transplant (not including advanced age or extensive prior therapy).
AML—acute myelodysplastic syndrome; BMT—bone marrow transplant; CML—chronic myelogenous leukemia. MDS—myelodysplastic syndrome.

(2-CDA) and cytarabine. These are established myeloid leukemia induction chemotherapy regimens that are only moderately myelosuppressive and can be safely administered without transplantation in elderly patients [41,42]. Allogeneic cells were infused 2 days after the last dose of chemotherapy. All patients were treated on an in-patient basis with standard supportive care [36•]. Prophylactic treatment for GVHD generally included tacrolimus and mini-dose methotrexate [43].

We treated 46 patients with AML, CML, and MDS (myelodysplastic syndrome) who were considered ineligible for ablative treatment because of advanced age (>55 years) or organ dysfunction. Table 2 summarizes the

patient characteristics. The median age was 60 years (range, 29 to 75). Twenty-six patients were aged 60 years or older, and seven were 70 years or older. Twenty-seven had medical comorbidity including coronary heart disease (four patients), arrhythmia (two patients), congestive heart failure or ejection fraction less than 50% (six patients), cerebrovascular disease (three patients), and recent or active treatment of opportunistic infections (seven patients). The median donor age was 61 years (range, 24 to 76 years). Thirty-five patients received transplants from a complete HLA-matched sibling, nine from a one-antigen-mismatched related donor, and two from a matched unrelated donor. Peripheral blood stem cells were collected for the matched related transplants and bone marrow for the mismatched or unrelated transplants to avoid excessive risk of GVHD in that setting. No complications were reported for any of the collecting procedures. Stem cell collection was safe for elderly donors also [44].

Engraftment

Four patients died early and were not evaluable for engraftment. Thirty-six of 40 evaluable patients receiving related-donor transplants engrafted with donor cells. Absolute neutrophil count reached 0.5×10^9/L at a median of 13 days (range, 9 to 38), and platelet count reached 20×10^9/L at a median of 16 days (range, 8 to 78); however, four patients never achieved platelet transfusion independence. Upon engraftment, 23 patients achieved complete donor chimerism, and 13 were mixed chimeras. Three patients had autologous reconstitution of hematopoiesis, and one died of graft failure. Four of the initial mixed chimera patients later converted to complete donor chimeras, and two lost detectable donor cells and had autologous reconstitution. All the other patients with mixed chimeras, except two who are still too early to assess, experienced a relapse, and there were no long-term mixed chimeras. The two patients with CML receiving matched unrelated donor transplants did not achieve engraftment.

Toxicity and GVHD

Treatment-related mortality (TRM) occurred in eight patients: two died from diffuse alveolar hemorrhage, two from infections, one from graft failure, and three from acute GVHD. Severe toxicity (Bearman grade 3) [45], not contributing to death, occurred in four additional patients. The actuarial rate of TRM was 18 ± 6%. Grade 2–4 acute GVHD occurred in 11 patients, establishing an actuarial rate of 33 ± 8%. Grade 3–4 manifestations occurred in four patients and were fatal in three patients. Thirty patients achieved engraftment and survived beyond day 100, and seven of them developed chronic GVHD, with an actuarial rate of 44 ± 13%. No patients died of chronic GVHD, and it was generally relatively mild.

Outcomes

Thirty-five patients had AML or MDS. Patients were classified as low- or high-risk based on the status of their disease at transplant, although all the patients had poor prognosis based on age, adverse cytogenetics, antecedent hematologic disorder, or disease beyond first remission. The low-risk group included 17 patients: 10 with disease in first complete or partial remission (CR/PR) and seven in a second or third CR/PR. PR was defined as chemosensitive disease with under 10% blasts in the marrow and no peripheral blasts. The high-risk group included patients with refractory disease or high leukemia burden. Overall, 28 of 31 evaluable patients achieved CR. Fifteen patients later relapsed, and three died in remission. Six of the relapsing patients received DLI, but none responded. Six patients received a second non-ablative transplant with the alternate regimen or with a melphalan-based regimen, and two remain in continuous remissions. In all, 14 patients are alive, with a median follow-up of 9 months, and 12 are currently disease-free, two after additional immune-based therapies. The median survival of the whole group was 6.5 months; and 40 ± 9, (25 ± 10%) are alive at 1 and 2 years after transplant. The relationship of outcome to disease status at transplant was significant (Fig. 1). The median survival of the low risk-group has not been reached, and 1- and 2-year survival rates were 78 ± 12% and 58 ± 19%. One and 2-year disease-free survival (DFS) rates were 57 ± 14% and 19 ± 16%. The current DFS rate (considering patient responding to additional immune-based treatments as disease-free) [46] was 60 ± 15% and 40 ± 19% at 1 and 2 years, respectively (Fig. 2). These are encouraging results considering the poor prognosis of these patients with conventional chemotherapy. The outcome of the high-risk group was grim. Overall survival and disease-free survival were 11 ± 7% and 6 ± 5% 1 year after transplant, respectively.

Eleven patients had CML, seven in late chronic phase and four in accelerated phase or second chronic phase. Two patients receiving matched unrelated transplants did not achieve engraftment. Eight of the remaining patients achieved complete hematologic and cytogenetic remission. The three patients with accelerated-phase disease at transplant relapsed and died, with a mean survival of 22 months. Of the six patients with chronic-phase disease receiving a related-donor transplant, two died of GVHD, and three (ages 47, 63, and 67 years) are currently disease-free 4, 22, and 19 months after transplant, respectively. The third patient received a DLI for an early relapse and remains in molecular CR 15 months after the procedure. One patient refused DLI and is alive with residual CML. Overall survival of the patients with chronic-phase disease at transplant was 67 ± 19% in 2 years, and the DFS is 50 ± 20%. Figures 1 and 2 show the probabilities of survival and current disease-free survival of AML/MDS and CML patient groups combined together and divided by risk.

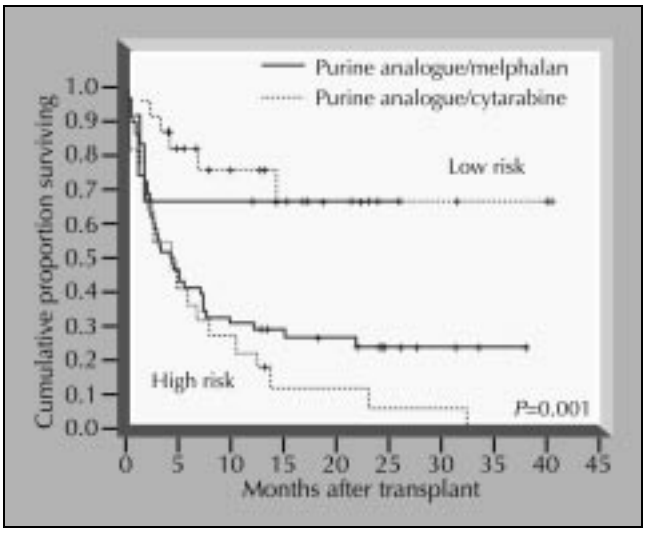

Figure 1. Probability of survival (Kaplan–Meier curve) of 116 patients by disease status at transplant and preparatory regimen. "Low risk" is defined as disease in remission or chemosensitive disease with low leukemia load (no peripheral blasts and less than 10% bone marrow blasts) or CML in chronic phase. "High risk" is defined as disease in relapse or CML beyond chronic phase.

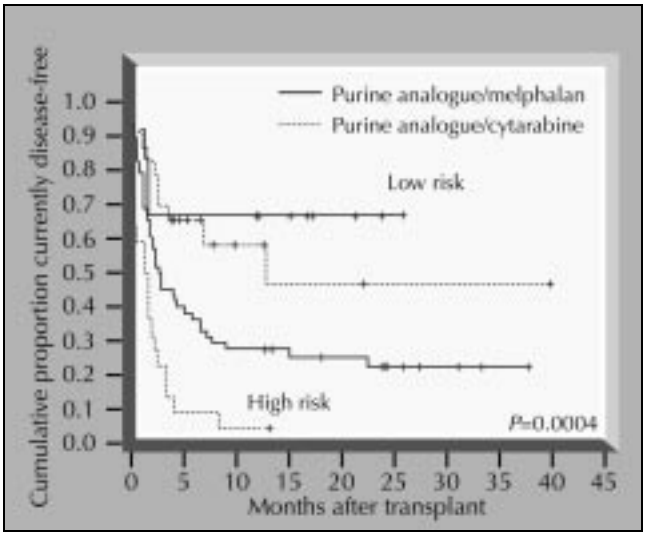

Figure 2. Probability of current disease-free survival (Kaplan–Meier curve) of 116 patients by disease status at transplant and preparatory regimen. Patients who relapsed after transplant but entered and remained in remission after immune-based therapies are considered currently disease free. Risk is defined as in Figure 1.

Purine Analogue/Melphalan-containing Non-ablative Regimens

The outcome of patients with active chemotherapy-refractory leukemia at time of transplant with the purine analogue/cytarabine-containing regimens was poor. Although most patients initially achieved remission, they relapsed shortly afterwards and died of their disease. Presumably, the disease progressed in these patients before their development of an effective GVL response, or drug-resistant leukemia is also resistant to immunologic mechanism. This regimen also seemed not to be immunosuppressive enough to allow engraftment of transplants from unrelated donors. The combination of melphalan with purine analogues was subsequently explored in an effort to provide better antileukemic effect without undue toxicity [47].

Melphalan has a broad spectrum of activity in a variety of hematologic malignancies, including myeloid leukemia [48]. It is well tolerated, with little extra-medullary toxicity. There is also synergism with purine analogues, which have been shown to inhibit the mechanism of DNA repair after alkylating agent–induced damage [49]. The conditioning regimens we used are outlined in Table 1. We used melphalan in combination with fludarabine or cladribine. The fludarabine/cladribine arm of the study was closed after enrollment of six patients because of excessive renal toxicity. This regimen is more intensive then the purine analogue/cytarabine combination and is usually given with stem cell support [50]. It is non-ablative, but delayed recovery of hematopoiesis might occur in the absence of stem cell support.

We have treated 70 patients, 43 with AML/MDS and 27 with CML. The median age was slightly lower than in the purine analogue/cytarabine group and was 54 (24 to 70) years. This study included 32 patients receiving matched unrelated transplants. All the patients were considered ineligible for conventional transplant because of age (>55 for related and >50 for unrelated transplants), extensive prior therapy, prior BMT (17 patients), or organ dysfunction. As with the purine analogue/cytarabine group, 83% of the patients were considered at high risk because of disease that was not in remission at the time of transplant or disease that was beyond the first chronic phase for patients with CML. Sixty-four patients were evaluable for engraftment. One failed to achieve engraftment, and one additional patient had secondary graft failure.

Acute grade 2–4 and 3–4 GVHD occurred in 44 ± 7% and 19 ± 5% of the patients, respectively. The rates for the patients who underwent related transplants were 28 ± 8% and 12 ± 6%, and these rates are similar to those observed in the purine analogue/cytarabine study. The matched unrelated transplants resulted in higher GVHD rates: 60 ± 9% and 32 ± 9% of the patients developed grade 2–4 and 3–4 disease, respectively (P=0.02). Thirty-eight patients survived at least 100 days, and 17 developed chronic GVHD, with an actuarial rate of 67 ± 10%. This finding was similar in the related and unrelated groups. Transplant-related mortality within the first year occurred in 30 patients, with an actuarial rate of 45 ± 10%: 54 ± 9% for the matched unrelated transplants and 34 ± 8% for the related-donor transplants (P-value not significant). The toxicity of this regimen was higher than that of the purine analogue/cytarabine regimen (P=0.008).

Twenty-three patients are alive, with a median follow-up of 22 months (range of 12 to 38). The median survival of the whole group was 4.5 months; 37 ± 6% and 31 ± 6%

survived after 1 and 2 years, respectively. Again, disease status at transplant had a major impact on outcome (Fig. 1). Estimated survival rates at 1- and 2-year follow-up after transplant in the low-risk group, as defined above, were 67 ± 14% and 57 ± 15%, respectively, and 31 ± 6% and 24 ± 6% in the high-risk group (P=0.03). One- and 2-year DFS rates followed the same pattern: 67 ± 14% and 57 ± 15% for the low-risk group, and 26 ± 6% and 23 ± 6% in the high-risk group, respectively (P=0.03)(Fig. 2). Survival rates were similar for the AML/MDS and the CML groups. Younger patients tended to have better outcome but not reaching statistical significance. Matched unrelated donor and related-donor transplants were not different in outcome. Extensive prior therapy and adverse cytogenetics predicted a worse outcome, but prior BMT did not. Once again, patients surviving at least 100 days had a better relapse-free survival rate if they developed acute or chronic GVHD. Most long-term survivors had experienced GVHD. This more intensive purine analogue/melphalan preparative regimen was more effective in refractory disease, with reduced risk of relapse, presumably providing longer disease control and allowing time for development of GVL effects.

Other Approaches for Non-myeloablative Transplants

Slavin *et al*. [39•] used a similar approach in a study employing a regimen combining fludarabine and busulfan at 50% of the conventional ablative dose (8 mg/kg) and antithymocyte globulin. They used short-term GVHD prophylaxis with cyclosporine for 100 days. This regimen has reduced toxicity compared with the higher-dose busulfan/cyclophosphamide regimens, but still requires blood stem cell or marrow transplantation for prompt hematopoietic recovery. In an updated report, a total of 48 patients were treated, ages 1 to 63 years. Twenty-three had myeloid malignancies, and the others had lymphoid malignancies or benign diseases. Engraftment was documented in all patients. Severe GVHD occurred in eight of 40 patients with malignant diseases. Seven patients relapsed, and some responded to DLI. Overall, 32 of 40 patients remained alive at the time of the report. The same regimen was successful in achieving engraftment of HLA-mismatched related and matched unrelated donor grafts as well. This regimen produces marked myelosuppression and has not been administered before without marrow support. The regimen was well tolerated in a group of younger patients, and the tolerance of elderly patients is currently been tested.

Childs *et al*. [25] recently reported their experience with a non-ablative regimen consisting of fludarabine and cyclophosphamide. Fifteen patients with a variety of hematologic and non-hematologic malignancies have been treated. Graft-versus-malignancy effect was observed in some of the patients. We have used a similar regimen successfully in patients with low-grade lymphoid malignancies.

Following the results obtained from their preclinical canine model, Storb *et al*. [51] and McSweeney *et al*. [52] developed a non-ablative strategy for elderly patients. Their preparative regimen consists of low-dose total-body radiation and pre- and posttransplant immunosuppression with cyclosporine and mycophenolate–mofetil to prevent GVHD and graft rejection. DLI is given to convert mixed chimeras to complete donor chimeras. A preliminary report of results with eight patients treated with this strategy showed low toxicity and establishment of mixed chimerism in all patients; however, follow-up time is still short for assessment of outcome. Other reduced-toxicity regimens have been proposed [53,54].

Conclusions

Non-myeloablative allogeneic hematopoietic transplants are feasible in elderly patients and patients with comorbidities precluding standard ablative conditioning with acceptable toxicity. Engraftment with donor cells occurs in most patients, but a more intensive regimen is needed for engraftment of unrelated marrow or in immunocompetent or sensitized patients. Mixed chimerism occurred early in the course in some patients but was not stable in these leukemic patients. Some mixed chimeras converted to either autologous or complete donor-derived hematopoiesis, and most of the others relapsed.

Favorable outcomes occurred in the patients with disease in remission or with low leukemia burden at the time of transplant with both regimens. Sixty to seventy percent are alive 2 years after transplantation. These are encouraging results considering the poor prognostic features of the patients treated, such as age, adverse cytogenetic abnormalities, prior hematologic disorder, and disease beyond first remission. Patients with refractory disease tended to have better disease control with the more intensive purine analogue/melphalan regimen (P=0.01), with an estimated survival rate of 24 ± 6 % at 2 years; however, this regimen was also more toxic. This suggests that a more intensive conditioning may be necessary in the setting of high leukemia burden to allow time for the development of an effective GVL effect.

Patients who developed acute GVHD had a lower probability of survival in the first 100 days posttransplant, but for those surviving this period, a history of acute and/or chronic GVHD was associated with higher probability of long-term disease control (Fig. 3). Most long-term disease-free survivors had GVHD, a finding that is consistent with the known association of GVHD and GVL. Control of GVHD remains a major obstacle to successful hematopoietic transplantation, and development of techniques to separate GVL and GVHD is critical for further development of this strategy.

The optimal intensity of the preparative regimen depends on several factors, including patient age, aggressiveness and chemosensitivity of the underlying malignancy, its sensitivity to the GVL effect, immunocompetence of the

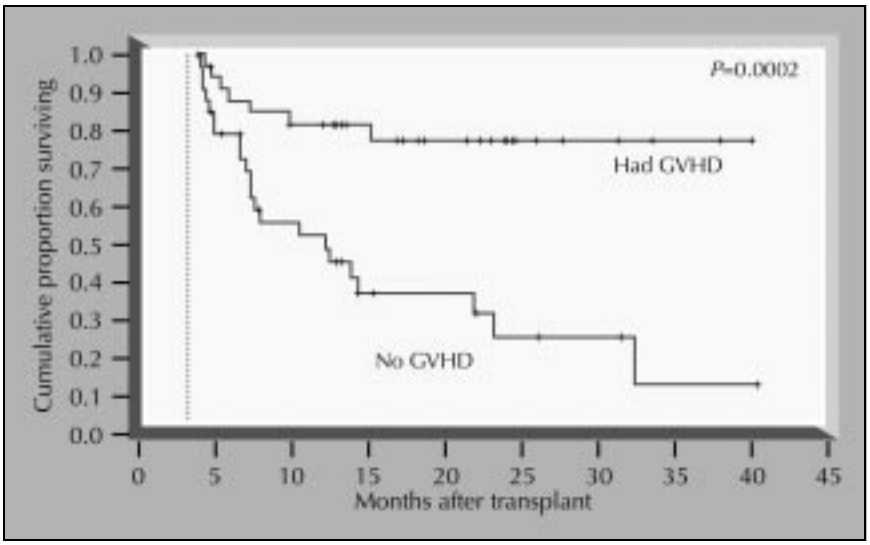

Figure 3. Probability of survival (Kaplan–Meier curve) of 68 patients surviving at least 100 days after transplant by history of acute and/or chronic graft-versus-host disease.

recipient, and genetic disparity between the donor and recipient. Patients with acute myelogenous leukemia require at least short-term disease control to allow development of graft-versus-malignancy effects. This may not be necessary in chronic myelogenous leukemia given its more indolent course and greater sensitivity to graft-versus-leukemia effects.

Further clinical trials are required for prospective comparison of this approach with other chemotherapy techniques in elderly patients or those considered ineligible for conventional myeloablative transplant because of other comorbidities. Elderly patients can be considered for clinical trials and should not be denied transplantation-based treatment because of age alone. Theoretically, non-ablative regimens can also produce superior results in younger patients who are candidates for conventional regimens, but the answer to this question will require carefully planned prospective comparative trials.

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