

Graft-versus-tumor effects

Myeloablative allografting for chronic lymphocytic leukemia: evidence for a potent graft-versus-leukemia effect associated with graft-versus-host disease

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Summary:

In all, 30 patients with CLL proceeded to myeloablative allogeneic BMT using related ($n = 20$, 67%) or unrelated ($n = 10$) donors, at the Princess Margaret Hospital (Toronto) ($n = 20$) or the Leukemia/BMT Program of BC (Vancouver) ($n = 10$), from 1989 to 2001. Median (range) interval from diagnosis to BMT was 4.8 (0.3–13) years, median number of prior therapies was three and median age 48 years. The preparative regimen included total body irradiation in 15 (50%). In all, 14 of 30 patients (47%) are alive, with median (range) follow up of 4.3 (2.4–10.5) years. All are in complete remission, two following therapy for post-BMT progression. Actuarial overall (OS) and event-free survival (EFS) at 5 years is 39% (OS 48% for related donor and 20% for unrelated donor BMT); cumulative incidence of nonrelapse mortality (NRM) and relapse is 47 and 19%, respectively. Both acute (RR = 0.008, $P = 0.01$) and chronic (RR = 0.006, $P = 0.02$) Graft-versus-host disease (GVHD) were associated with markedly decreased risk of relapse. Patients receiving grafts from unrelated donors had increased NRM (RR = 3.6, $P = 0.02$) and decreased OS (RR of death = 3.4, $P = 0.002$). Allogeneic BMT has resulted in long-term EFS in approximately 40% of patients with CLL. There is evidence for a strong graft-versus-leukemia effect associated with acute and chronic GVHD, resulting in near complete protection from relapse.

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Chronic lymphocytic leukemia (CLL) is a B-cell leukemia of undetermined etiology and variable clinical course that it is not uncommon, but remains incurable with conventional therapies.^{1,2} Myeloablative allogeneic hematopoietic stem cell transplantation (HSCT) can be employed with curative intent in CLL. Published series to date^{3–12} have established feasibility, but there remains a paucity of mature data.^{13,14} Long-term analysis of results that may be achieved with this transplant modality is therefore important, and will permit comparisons with outcomes from other therapies, high dose¹⁴ and conventional as well as more recent experience with nonmyeloablative transplants.¹⁵ Results are herein presented of the combined experience of two large Canadian transplant centers (Princess Margaret Hospital, Toronto, Ontario, and the Leukemia/BMT Program of BC, Vancouver, BC) to examine the impact of myeloablative allogeneic HSCT on disease progression and long-term survival in CLL.

Patients and methods

Between 1989 and 2001, 30 patients proceeded to allogeneic BMT at the Princess Margaret Hospital ($n = 20$) and the Leukemia/BMT Program of BC ($n = 10$) for CLL. Preliminary data for some of the Vancouver patients were previously reported;^{10,16} results from the entire group are presented here with a median follow-up of 4.3 (range 2.4–10.5) years. Eligibility criteria included failure of one or more conventional treatment regimens, and demonstration of adequate organ function. Response to last prior therapy was not a prerequisite for BMT.

Patient characteristics

The diagnosis was B-cell CLL in all patients. Of the patients, 18 (60%) were male and 12 female. Median age

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Table 1 Patient characteristics (*n* = 30)

Age in years, median (range)	48 (32–59)
Sex (male/female)	18/12
Interval from diagnosis to BMT, years, median (range)	4.8 (0.3–13)
<i>Diagnosis, n = (%)</i>	
B-cell CLL	30 (100)
<i>Stage^a, n = 0</i>	
I	3
II	6
III	5
IV	3
<i>Prior therapy, n = (%)</i>	
Alkylating agents	27 (90)
Nucleoside analog therapy	20 (67)
Multiagent chemotherapy	19 (63)
Radiotherapy	0
Number of regimens, median (range)	3 (1–10)
<i>Institution, n =</i>	
Princess Margaret Hospital	20
Leukemia/BMT Program of BC	10

^aAt diagnosis, Rai stage.

(range) at BMT was 48 (32–59) years. Patient characteristics are summarized in Table 1.

The majority (21 patients, 70%) were Rai stage II or higher at diagnosis; 13 (43%) were Rai stage IV. Median interval from diagnosis to transplant was 4.8 (0.3–13) years. Prior therapy included nucleoside analogs in 20 patients (67%), and multiagent chemotherapy in 19 (63%). In almost all (27/30, 90%) prior alkylating agents had failed. A median of three (range 1–10) prior therapies had been administered, with 18/30 (60%) of patients having received three or more therapies. No patient received radiotherapy prior to stem cell transplant conditioning.

Transplant features

All patients received marrow harvested from the pelvis. The donor was an HLA-matched sibling for 20 patients (67%), and an HLA-matched unrelated volunteer for 10 patients (33%). The conditioning regimen included cyclophosphamide and total body irradiation ± other agents in 15 (50%) of patients, and busulfan plus cyclophosphamide in 15 (50%). Graft-versus-host disease (GVHD) prophylaxis consisted of cyclosporine and methotrexate in the majority (28 patients, 93%); T-cell depletion was included in two cases.¹⁷ Transplant features are summarized in Table 2.

Pretransplant workup was as appropriate to ensure adequate organ function and to establish disease status. Diagnosis was established by standard morphologic and immunophenotypic criteria¹⁸ on peripheral blood and marrow samples by experienced hematopathologists in the two programs. Response was assessed using National Cancer Institute Guidelines.¹⁸ This cohort spans 13 years and therefore full information is not available for the more recently recognized prognostic factors such as immunoglobulin gene mutation profile, genomic aberra-

Table 2 Transplant features (*n* = 30)

	<i>n = (%)</i>
<i>Conditioning regimen</i>	
Cyclophosphamide + fractionated total body irradiation ± other	15 (50)
Busulfan + cyclophosphamide	15 (50)
<i>Donor marrow</i>	
HLA-matched sibling	20 (67)
Unrelated donor	10 (33)
<i>GVHD prophylaxis</i>	
Cyclosporine + methotrexate	28 (93)
T-cell depletion ± other	2 (7)

tions or ZAP-70 status.^{19–24} Patients provided written informed consent.

Supportive care

All patients were hospitalized for the conditioning regimen and transplantation procedure. All Vancouver patients were nursed in HEPA-filtered rooms, as were Toronto patients from 1996 onwards. Infection prophylaxis included acyclovir ± ganciclovir ± i.v. Ig, trimethoprim sulfamethoxazole or pentamidine for *Pneumocystis carinii* prophylaxis, heparin for veno-occlusive disease prophylaxis (Vancouver only), and fluconazole or low dose amphotericin as per sequential institutional policies (Vancouver only). All blood products were irradiated.

Statistical analysis

Overall (OS) and event-free survival (EFS), nonrelapse mortality (NRM), acute and chronic GVHD, and refractory/recurrent disease were the primary outcomes of interest. Survival curves were plotted where appropriate using the methods of Kaplan and Meier.²⁵ Probabilities of acute and chronic GVHD, NRM, and refractory/recurrent disease were determined using cumulative incidence estimates.²⁶ Univariate and multivariate analyses (UVA and MVA) were performed to examine for pre- and post-transplant factors associated with the outcomes of interest. Cox proportional hazards regression was employed, with time-dependent covariates. Impact of prior therapy on development of acute and chronic GVHD was assessed. Age, gender, stage, interval from diagnosis to BMT, center (Toronto vs Vancouver), year of BMT (1989–1995 vs 1996–2001) prior nucleoside analogs, prior multiagent chemotherapy, conditioning regimen, donor type and development of acute or chronic GVHD were tested as applicable for effect on the outcome variables – OS, EFS, relapse, NRM, and acute and chronic GVHD.

Results

Outcome

Out of 30 patients 14 (47%) are currently alive at a median follow-up time of 4.3 years (range 2.4–12.8). All surviving

patients are in complete remission (CR), two following successful therapy for relapsed disease. In all, 16 patients (53%) are deceased, a median (range) of 1.4 (0.04–4.5) years post-BMT, 13 with nonrelapse causes and three with progressive chronic leukemia.

OS is 39% (95% CI 20–59) (Figure 1) and EFS 39% (95% CI 21–58). OS and EFS did not differ significantly between the two centers ($P=0.3$, log rank). No deaths/events/relapses have occurred more than 5 years post BMT. Follow-up is complete for all surviving patients.

Survival analysis demonstrated an improved OS for related vs unrelated donor marrow source at 48% (95% CI 20–72) vs 20% (95% CI 3–47), $P=0.03$ (Figure 2) and EFS at 60% (95% CI 36–78) vs 10% (95% CI 0.6–36%), $P=0.01$.

In MVA unrelated donor graft had a negative impact on OS (relative risk (RR) of death = 3.4, $P=0.02$) and EFS (RR = 3.7, $P=0.02$) when compared with related donor graft. This was not clearly mediated entirely by GVHD, as no significant difference was noted between type of donor with regard to incidence and severity of acute or chronic GVHD (see GVHD section). Cause of death in the unrelated donor setting included infection (3), GVHD (3), and regimen related (2). Two of 10 patients (20%) who received marrow from an unrelated donor are alive, compared with 12 of 20 patients (60%) who received related donor marrow. However, the two surviving unrelated donor patients are 5 and 8 years post-BMT, in CR.

Results did not significantly differ between the two centres (all P -values > 0.2), permitting rational data combination. Outcomes were similar for patients transplanted prior to vs after 1996; however, the majority ($n=24$ or 80%) were transplanted between 1996 and 2001. Significant predictive factors are summarized in Table 3.

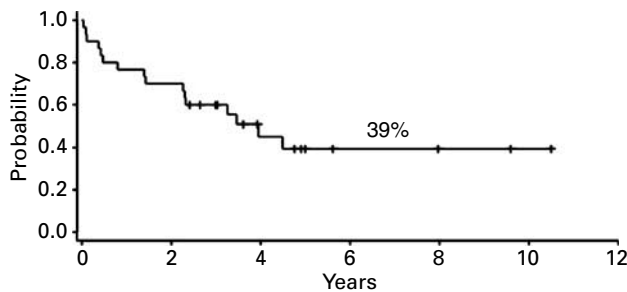


Figure 1 Probability of OS following allogeneic BMT for CLL ($n=30$).

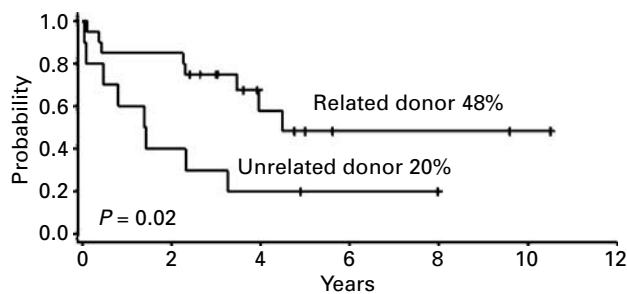


Figure 2 Probability of OS following allogeneic BMT for CLL ($n=30$) using related ($n=20$) and unrelated donors ($n=10$).

Non relapse mortality

Death occurred from causes other than relapse in 13 patients, a median of 509 (range 13–1640) days post-BMT, including 10 from GVHD and/or infection, two from regimen-related toxicity and one from myocardial infarction. Unrelated donor stem cell source was the only factor significantly associated with NRM in univariate (RR of death = 3.4, $P=0.03$) and MVA (RR = 4.2, $P=0.01$) (Table 3). The cumulative incidence (95% CI) of NRM was 47% (26–66%) (Figure 3).

Table 3 Univariate and multivariate analysis

Significant predictive factors ^a	Univariate		Multivariate	
	RR	P-value	RR	P-value
<i>Overall survival^b</i>				
Unrelated donor marrow source	2.9	0.03	3.4	0.02
<i>EFS^c</i>				
Unrelated donor marrow source	3.2	0.02	3.7	0.02
<i>Relapse</i>				
Acute GVHD Gr. 2–4	0.008	0.01	0.008 ^d	0.01
Chronic GVHD	0.006	0.02	0.006 ^d	0.02
<i>Nonrelapse mortality</i>				
Unrelated donor marrow source	3.4	0.03	4.2	0.01
<i>Chronic GVHD</i>				
Prior nucleoside analogs	3.1	0.05	NS	NS
Prior multiagent chemotherapy	0.3	0.03	0.2	0.003
<i>Acute GVHD</i>				
No significant predictive factors				

^aAge, gender, stage, interval from diagnosis to BMT, center, year of BMT, number of lines of prior therapy, prior nucleoside analogs, prior multiagent chemotherapy, conditioning regimen, donor marrow source, and development of acute or chronic GVHD were tested as applicable for effect on the outcome variables.

^bRelative risks here indicate risk of death.

^cRelative risks here indicate risk of event: death or relapse.

^dBoth acute and chronic GVHD retained significance in MVA models testing other factors; the relationship between acute and chronic GVHD in combination could not be explored in this analysis.

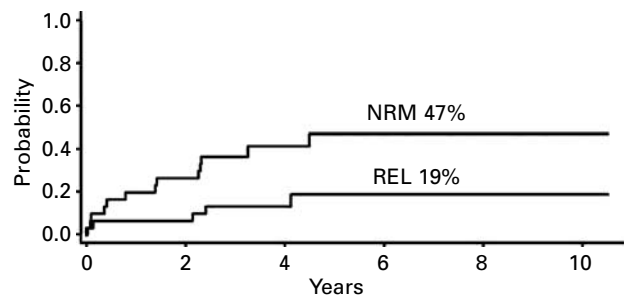


Figure 3 Cumulative incidence of nonrelapse mortality (NRM) and relapse (REL) following allogeneic BMT for CLL ($n=30$).

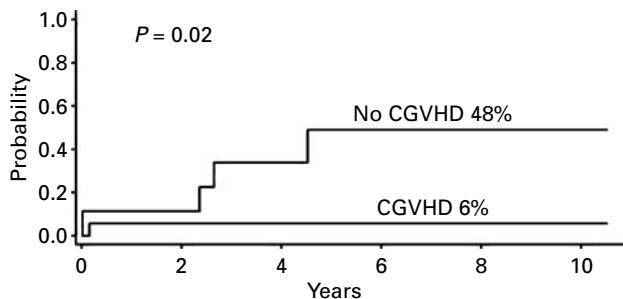


Figure 4 Cumulative incidence of relapse following allogeneic BMT for CLL ($n=30$) for patients surviving to day +100 ($n=26$) who did (CVGHD) or did not (no CGVHD) develop chronic GVHD.

Refractory/progressive leukemia

Five patients had refractory/progressive CLL a median of 784 (range 3–1504) days post BMT. Relapse caused death in one patient and two died from causes other than disease (infection one, myocardial infarction one). Two patients have achieved CR with further therapy, and are now alive and disease free 4 and 8 years after initial BMT. One patient had graft failure following T-cell depleted unrelated donor BMT, and subsequent recovery of oligoclonal host hematopoiesis after a second (T-cell replete) BMT from the same unrelated donor. CAMPATH salvage treatment was used successfully for progressive CLL, which occurred on day +1504 following the first BMT. It is now more than 1 year after this patient received a third stem cell transplant from a different unrelated donor at another institution, and she is alive and free of disease more than 8 years following the original transplant. The second patient was successfully managed with interleukin-2 and his course has recently been reported.²⁷ No donor leukocyte infusions were administered to these patients.

The cumulative incidence (95% CI) of refractory/progressive leukemia was 19% (9–37%) (Figure 3). In UVA, occurrence of acute grade 2–4 (RR of relapse = 0.008, $P=0.01$) and chronic GVHD (RR = 0.006, $P=0.02$) were each found to be associated with a decreased risk of relapse. Both remained significant in MVA testing for independent effect of other pre- and post-transplant factors on relapse. It was not possible to separate the specific independent contribution of the acute vs chronic GVHD in this study.

Chronic GVHD had a significant and positive impact on risk of relapse. Near complete protection from relapse was seen in patients surviving past day +100 who developed chronic GVHD. The cumulative incidence of relapse was 6% in this group (95% CI 0–23) vs 48% (95% CI 10–79) in those who did not develop chronic GVHD ($P=0.02$, Figure 4).

Graft-versus-host disease

The cumulative incidences of acute GVHD grades II–IV, and III–IV, and chronic GVHD were 52% (95% CI 32–68); 7% (95% CI 1–19), and 65% (95% CI 43–81) respectively.

No significant factors were found that predicted development of acute GVHD, including unrelated donor marrow source or conditioning regimen ($P>0.3$ for all). No association was seen between prior use of nucleoside analogs, multiagent chemotherapy or radiotherapy, and development of acute GVHD. Prior nucleoside analog use was significantly associated with an increased risk of chronic GVHD in UVA (RR = 3.1, $P=0.05$), but this effect was nonsignificant after inclusion of prior multiagent chemotherapy in the MVA. Prior multiagent chemotherapy was associated with a decreased probability of chronic GVHD in both UVA and MVA (RR = 0.2, $P=0.002$ MVA) (Table 3). Number of prior therapies was not found to influence acute or chronic GVHD. No statistical difference in risk of development of acute GVHD grade 2–4; 3–4, or chronic GVHD was seen when related and unrelated donors were compared.

Discussion

These results demonstrate achievement of long-term disease-free survival and presumably cure in a significant proportion (40%) of patients with CLL after myeloablative allogeneic BMT, including half of those with a sibling donor. Allografting is a unique therapy for this disease, as the only one to date that has resulted in durable remission. This experience confirms and extends prior positive preliminary reports^{3–13} in a large group of patients, with a long median follow up, approaching 5 years. Lead patients are now disease free for approximately a decade. A strong and positive effect of both acute and chronic GVHD, and presumably graft-versus-leukemia (GVL), in preventing relapse post allograft in CLL was seen in this analysis.

The cumulative incidence of refractory/progressive CLL in this series is low, less than 20%. This is an encouraging result in a malignancy where cure has not been demonstrated by a variety of sequential conventional therapies. The latest relapse occurred just over 4 years post allograft. Moreover, five of 14 surviving patients are alive in CR more than 5 years post transplant, and are likely to have been cured of their disease. The incidence of relapse was particularly low at 6% in patients with chronic GVHD.

The apparent plateau in the survival curve is in contrast to results seen to date with autografting in CLL, whereby the incidence of relapse is high, and it is not yet clear if survival prolongation may be achieved.^{28,29} However, as with autografting, it is possible that prognostic factors play a role in determining which patients may benefit from this therapy.^{14,29,30}

One-third of the patients in this series received marrow from unrelated donors. These patients had a significantly poorer overall survival related to an increase in NRM. Although the incidence of acute and chronic GVHD did not differ significantly between the related and unrelated donor patients, death was typically related to infection and GVHD in the unrelated donor setting. However, 20% of these patients survive 3–8 years post transplant, free of disease, indicating that the outcome is promising for patients who survive the high risk early period. Results are sufficient to warrant reservation of myeloablative unrelated donor HSCT

for patients with CLL who are expected to do poorly with other available therapeutic modalities.

An early report suggested that the incidence of GVHD is lower in patients who have received nucleoside analogs such as fludarabine prior to allogeneic HSCT.⁵ These data are supported mechanistically by experiments in mice.³¹ In this series, we have observed the reverse, namely that the incidence of chronic, but not acute GVHD is statistically significantly higher in patients who had received fludarabine as part of their prior therapy. Similarly, Vancouver results in follicular lymphoma allografts have failed to demonstrate a significant decrease in GVHD for patients who have received prior fludarabine treatment.³² The reasons for these discrepant results are not clear, but may reflect differences in timing and dosing of fludarabine, and other pre- and post-transplant factors that independently influence the incidence of acute and/or chronic GVHD.

The Seattle group found that patients who received TBI as part of their preparative regimen for allografting in CLL fared better than those who did not.¹¹ In this series, 50% of patients received TBI, in part due to institutional preferences for preparative regimen (Vancouver – TBI; Toronto – busulfan). In UVA and MVA use of TBI in the conditioning regimen was not found to impact significantly on overall or event-free survival, risk of NRM, relapse, acute or chronic GVHD.

Previous reports have supported the existence of a GVL effect in allogeneic HSCT in CLL.^{14,33–40} The positive impact of GVHD/GVL on relapse post allograft for CLL is convincingly demonstrated in this analysis. The principle of the antitumor effect of GVH/GVL is employed in the strategy of nonmyeloablative allografting. Multiple series have reported promising early results using this strategy for CLL.^{15,37–49} Safety and feasibility have been demonstrated, along with some evidence of disease control. Although regimen-related toxicity is lower in nonmyeloablative as compared with myeloablative allografting,^{15,38} rates of GVHD do not appear dissimilar.⁵⁰ Recent series suggest the improvement in TRM is offset by an increased risk of leukemia progression.^{15,38,40} Best results appear to be achieved with responding disease that is in good control at the time of nonmyeloablative transplant, presumably allowing the GVL effect time to impact on disease control.^{38,44} Longer follow-up will be required to determine the stability of these outcomes.

In conclusion, myeloablative allogeneic BMT has resulted in long-term disease-free survival in ~40% of this cohort of patients with CLL, including half of those with a sibling donor. Both acute and chronic GVHD provided significant protection from relapse. These mature data may be compared to results achieved with nonmyeloablative allografting, in order to determine optimal therapy for patients with CLL.

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