Bone Marrow Transplantation for Chronic Myeloid Leukemia With Volunteer Unrelated Donors Using Ex Vivo or In Vivo T-Cell Depletion: Major Prognostic Impact of HLA Class I Identity Between Donor and Recipient

By Andrew Spencer, Richard M. Szydlo, Paul A. Brookes, Edward Kaminski, Simon Rule, Frits van Rhee, Katherine N. Ward, Geoff Hale, Herman Waldmann, Jill M. Hows, J. Richard Batchelor, and John M. Goldman

Between August 1985 and July 1994, we performed 115 volunteer unrelated donor (VUD) bone marrow transplants (BMT) for first chronic phase (n = 86) or advanced phase (n = 29) chronic myeloid leukemia (CML). Standard serologic HLA typing of potential donors and recipients was supplemented with one-dimensional isoelectric focusing (IEF) for class I proteins, allotyping for DR and DQ alleles using DNA restriction fragment length polymorphism (RFLP) analysis, and the measurement of antirecipient major histocompatibility complex (MHC) cytotoxic T-lymphocyte precursor cells in the donors' blood (CTLp assay). Recipients were conditioned for transplantation with a combination of high-dose chemotherapy and total body irradiation (n = 103) or high-dose chemotherapy alone (n = 12). Twenty eight recipients received ex vivo T-cell–depleted marrow, and 84 underwent some form of in vivo T-cell depletion. The probability of severe (grades III or IV) acute graft-versus-host disease (aGVHD) was 24%, and that of extensive chronic graft-versus-host disease (cGVHD), 39%. Proportional hazards regression analysis showed an association between low frequency CTLp and a reduced incidence of severe aGVHD (relative risk (RR), 0.28; P = .0035). The probability of relapse at 3 years was 23%, with first chronic phase disease being independently associated with a lower risk of relapse (RR, 0.71; P = .01). The overall leukemia-free survival (LFS) at 3 years was 37%; the LFS for the first chronic phase and advanced phase recipients was 41% and 26%, respectively. First chronic phase disease (RR, 0.56; P = .063) and the combination of recipient cytomegalovirus (CMV) seronegativity and an IEF-matched donor (RR, 0.48; P = .011) were both associated with improved LFS. The probabilities of survival and LFS for patients under 40 years of age transplanted in first chronic phase from an IEF-matched donor were 73% and 50%, respectively. We conclude that VUD BMT is a reasonable option for patients with CML; when using ex vivo or in vivo T-cell depletion, optimal results are achieved in patients transplanted in chronic phase with marrow from donors without demonstrable class I HLA mismatch and a low CTLp frequency.

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MATERIALS AND METHODS

Patient population. A total of 118 consecutive patients with a diagnosis of CML underwent VUD BMT at the Hammersmith Hospital (London, UK) between August 1985 and July 1994 (86 in first chronic phase (CP), 21 in accelerated phase, eight in second chronic phase, and three in blast crisis). All patients were evaluated shortly before BMT to establish their disease status. For the purposes of analysis, the accelerated phase and second chronic phase patients were grouped together as advanced phase (AP) patients (n = 29). The blast crisis group patients were not included, as too few were transplanted to allow meaningful comparison with the other patient categories. Patient and disease characteristics for the study population are outlined in Table 1.

Selection of VUDs. Potential unrelated donors were identified through the two UK donor registries, the Anthony Nolan Research Centre and the British Bone Marrow and Platelet Donor panel, and through other international registries. The initial steps for such identification have been described elsewhere. The HLA data on potential donors were serologically checked in our laboratory. The serologically recognized specificities were defined as previously described, and donors showing the closest serologic match were typed by more discriminating techniques. These included one-dimensional isoelectric focusing (IEF) of HLA class I molecules (n = 92) as standardized by the 10th Histocompatibility Workshop, analyses for DR and DQ alleles by restriction fragment length polymorphism (RFLP; n = 104) as described by Bidwell et al, and limiting dilution analyses for cytoxic T-lymphocyte precursors (CTLp; n = 102). Donor-recipient pairs were classified according to CTLp into two groups, referred to as the high CTLp frequency (HF) group (n = 42) and the low frequency (LF) group (n = 60), with frequencies of greater than 1/100,000 and less than 1/100,000, respectively. A detailed description of the methodology for performing CTLp analysis has previously been published. Details regarding mismatches detected by serology, IEF, and RFLP, are outlined in Table 2. The final decision regarding the most appropriate donor for each patient was made on the basis of the typing results available at the time and donor and recipient cytomegalovirus (CMV) status.

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BLOOD, Vol 86, No 9 (November 1), 1995: pp 3590-3597
Recipient disease stage
1st CP  86
2nd CP   8
GVHD prophylaxis
EX-TCD  28
IN-TCD  84
Other    3
Patient/donor sex
M/M   34
M/F   29
F/M    21
F/F    31
Patient/donor CMV status
Negative/negative  41
Negative/positive  23
Positive/negative  23
Positive/positive  27
Enlarged spleen at DX
N  29
Y  78
Year of transplant
1985-1990  43
1989-1994  72
Median patient age, yr (range) 33.1 (4.1-51.9)
Median donor age, yr (range) 38.9 (22.1-55.8)
Median interval DX-TX, yr (range) 2.2 (0.9-6.8)
Median CTLp, 1:N × 10^3 (range) 149 (4-1,000)
Median leukocyte count at DX, x10^9/L (range) 200 (9.5-612)

Abbreviations: DX, diagnosis; TX, transplant; CTLp, cytotoxic T-lymphocyte precursor frequency in donor blood.

Pretransplant conditioning regimens. All recipients received antilymphocyte therapy with either antilymphocyte monoclonal antibodies (n = 110) or antilymphocyte globulin (n = 5) as part of their conditioning. The majority of patients (n = 103) received a combination of cyclophosphamide (60 mg/kg body weight daily) on days -6 and -5 and total body irradiation (TBI) on days -4 to -2, either 12 Gy (n = 31) or 13.2 Gy (n = 72), in six equal fractions. Additional total lymphoid irradiation (3 × 2 Gy on days -8 and -7) was administered to 23 recipients in combination with busulfan (8 mg/kg total dose; n = 11) or a single dose of daunorubicin (60 mg/m² body surface area; n = 12). The remaining 12 patients received a combination of cyclophosphamide (60 mg/kg body weight daily) on days -6 and -5, and busulfan (16 mg/kg total dose) on days -5 to -2. Thirty patients received splenic irradiation consisting of 10 Gy in two to four doses on days -8 and -7.

GVHD prophylaxis and management. The majority of patients either received marrow that had undergone ex vivo T-cell depletion (EX-TCD) with monoclonal antibodies (Campath-1M, n = 24; Campath-1G, n = 4) or in vivo T-cell depletion (IN-TCD) with varying forms of antilymphocyte therapy (Campath 1G, n = 78; antilymphocyte globulin, n = 2; Xomazyme, n = 4) after transplantation of donor marrow. Schedule details, including use of additional immunosuppression, are shown below. Intravenous cyclosporin A (CsA) was commenced at a loading dose of 5 mg/kg on day -3 and then reduced to 2.5 mg/kg/d thereafter. The dose was adjusted depending on weekly whole blood trough levels. In the absence of chronic GVHD, CsA was withdrawn by 12 months post-BMT. Methotrexate (MTX) was given at a dose of 8 mg/m² body weight on days 2, 4, 8, and 12 posttransplant. The final dose was withheld in the presence of severe mucositis. Schedule details were as follows: EX-TCD (n = 28); Campath 1M/complement (n = 15); Campath 1M/complement and CsA (n = 8); Campath 1M/complement and CsA/MTX (n = 1); or Campath 1G and CsA (n = 4). IN-TCD (n = 84); Campath 1G 5 mg twice daily intravenously, from days 1 to 5 and CsA/MTX (n = 78); antilymphocyte globulin 40 mL/d from days 1 to 5 (n = 2); or Xomazyme 0.1 mg/kg/d from days 1 to 5 (n = 4). Other (n = 3): CsA/MTX (n = 2); or CsA/MTX and low-dose methylprednisolone (n = 1).

Acute GVHD (aGVHD) was graded according to the Seattle criteria. Patients who developed grade III or IV aGVHD were deemed to be severely affected, whereas patients with grades 0, 1, and II disease were not. Acute GVHD of grades II to IV with rapid onset within 4 weeks of BMT was treated with methylprednisolone commencing at a dose of 1,000 mg/d intravenously. The rate of dose reduction depended on response. GVHD of more gradual onset or occurring later than 4 weeks from BMT was also treated with methylprednisolone but with a starting dose of 100 mg/d intravenously. Oral therapy was substituted as soon as possible. Refractory disease

Table 2. Donor-Recipient HLA Mismatches in 27 Patients

<table>
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<tr>
<th>UPN</th>
<th>Serology Mismatch (n=6)</th>
<th>IEF Mismatch (n=15)</th>
<th>RFLP Mismatch (n=15)</th>
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<tr>
<td>154</td>
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<tr>
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<td></td>
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<td>DR13/DR13</td>
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<tr>
<td>434</td>
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<td>DR16/DR15</td>
<td></td>
</tr>
<tr>
<td>436</td>
<td>DR10/DR15</td>
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</tbody>
</table>

Abbreviation: UPN, unique patient number.

* Limitations to determination of high resolution serologic definitions were occasionally experienced due to inadequate (leukemic) patient material and availability of local typing sera.

† RFLP HLA typing is based on clusters or combinations of fragments that constitute patterns specific for a single haplotype. They do not provide a basis for a formal designation, and we have, therefore, described RFLP patterns between donor/recipient pairs as being matched or mismatched, as determined by the molecular weights of the RFLP bands, for HLA-DR1 to HLA-DR18 phenotypes.
was treated with antilymphocyte monoclonal antibodies or antilymphocyte globulin. Chronic GVHD was graded as none, limited, or extensive.\textsuperscript{17} Treatment modalities included CsA, azathioprine, psoralen, and ultraviolet-A irradiation (PUVA).

Supportive care. All patients received broad spectrum oral antibiotics, amphotericin-B 100 mg four times a day by mouth, and amphotericin lozenges, commencing before BMT. As participants in a multicenter trial of prophylactic liposomal amphotericin B, eight patients received thrice weekly Ambisome (2 mg/kg; Vestar, Cambridge, UK). Since 1988, acyclovir 200 mg four times daily has been commenced before BMT and continued until day 35; since mid-1993, 12 patients who were CMV-seropositive and/or given marrow from a seropositive donor had prophylactic ganciclovir at 5 mg/kg intravenously 5 days per week substituted for the acyclovir as soon as possible after day 30, but not until neutrophil engraftment had occurred. Trimethoprim-sulfamethoxazole was commenced on day 30 post-BMT and continued until 1 year post-BMT, or longer in the presence of chronic GVHD or immunosuppressive therapy. Penicillin V was also commenced on day 30 post-BMT and continued indefinitely.

Virologic surveillance, from the time of BMT to day 120 post-BMT, of blood and urine for CMV (detection of early antigen fluorescent foci [DEAFF] test)\textsuperscript{18} and culture) and throat gargle for CMV had occurred. Trimethoprim-sulfamethoxazole was commenced on day 30 post-BMT and continued until 1 year post-BMT, or longer in the presence of chronic GVHD or immunosuppressive therapy. Penicillin V was also commenced on day 30 post-BMT and continued indefinitely.

Engraftment. Engraftment was recorded as occurring on the first of 3 consecutive days on which the peripheral blood neutrophil count was \( \geq 0.5 \times 10^9/L \). Establishment of donor hematopoiesis was confirmed by standard cytogenetic analysis. In the absence of a readily identifiable chromosomal marker, eg, in sex-matched Philadelphia chromosome (Ph)-negative CML transplants, alternative techniques including in situ hybridization and polymerase chain reaction (PCR) of variable number tandem repeats were performed. Engraftment failure was defined as a failure to establish donor hematopoiesis within 30 days of marrow infusion or loss of the graft after initially attaining satisfactory marrow function. Both instances were managed with reinfusion of previously cryopreserved autologous peripheral blood or bone marrow stem cells, with or without (usually without) additional immunosuppression.

Follow up. Detailed investigations, including bone marrow biopsy with cytogenetics and analysis of peripheral blood for BCR-ABL transcripts using competitive reverse transcription-PCR (RT-PCR),\textsuperscript{17} were performed at 3, 6, 12, 18, and 24 months, and then yearly thereafter. Competitive RT-PCR was performed more frequently if there was an increasing number of transcripts. For the purposes of analysis, relapse was defined as morphologic evidence of leukemia in the peripheral blood, marrow, or extramedullary sites. Nine patients have received leukocyte transfusions (DLT) from their original donor. Of these, five were in hematologic relapse, three had cytogenetic evidence of disease, and one patient had high numbers of transcripts on competitive RT-PCR (3,000 transcripts per microgram of RNA). Five of these patients have been reported previously.\textsuperscript{20} Three patients have undergone a second transplant from their original donor. The median follow-up time for the whole group was 284 days (range, 23 to 3,321 days) and for those surviving patients, 781 days (range, 108 to 3,321 days).

Clinical outcome. Infection. Patients were treated with antilymphocyte monoclonal antibodies or antilymphocyte globulin at 5 mg/kg intravenously 5 days per week substituted for the acyclovir as soon as possible after day 30, but not until neutrophil engraftment had occurred. Trimethoprim-sulfamethoxazole was commenced on day 30 post-BMT and continued until 1 year post-BMT, or longer in the presence of chronic GVHD or immunosuppressive therapy. Penicillin V was also commenced on day 30 post-BMT and continued indefinitely.

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Statistical methods. Probabilities of survival, leukemia-free survival (LFS), transplant-related mortality (TRM), graft failure, and developing GVHD were calculated by the method of Kaplan and Meier.\textsuperscript{21} The Lee-Desu statistic\textsuperscript{22} was used to test the association between prognostic variables and outcome. To determine whether any of the prognostic variables were independent predictors of outcome, those found to be significant at the \( P < .1 \) level in univariate analyses were examined using a proportional hazards regression analysis with a backward stepping approach. The prognostic variables considered are shown in Table 1, in addition to three factors describing matching/mismatching by serology, IEF, and RFLP. All detectable mismatches were included in the analysis; thus, the six serologic mismatches were also analyzed as IEF (\( n = 4 \)) or RFLP (\( n = 2 \)) mismatches. Based on disease status and matching by IEF, a subset of patients (\( n = 57 \)) was further analyzed by univariate methods to try to identify a cohort with the best possible LFS. Survival was assessed on the date of last patient contact and analyzed on October 31, 1994. All \( P \) values are two-tailed.

**RESULTS**

Engraftment. Sustained engraftment occurred in 99 of 115 recipients, with a probability of graft failure at 1 year of 16\% (95\% confidence interval [CI], 9\% to 25\%). The median time to neutrophil engraftment was 23 days (range, 12 to 42 days) and to achieve a platelet count of \( 50 \times 10^9/L \), 37 days (range, 17 to 270 days). Univariate analysis showed an increasing likelihood of graft failure with increasing donor age (\( P = .03 \); Table 3). Proportional hazards regression analysis showed an association between donor age of under 35 years and a reduced likelihood of graft failure (relative risk [RR] = 0.24; \( P = .03 \); Table 3). This was independent of the number of mononuclear cells infused. No other associations were demonstrable; specifically, there did not appear to be evidence of increased graft failure in those recipients who received HLA-mismatched, sex-mismatched, or ex vivo T cell-depleted marrow.

GVHD. The probability of severe aGVHD (grades III and IV) was 24\% (95\% CI, 17\% to 33\%). Univariate analysis showed an association between both IN-TCD (compared with EX-TCD; \( P = .03 \)) and high frequency CTLp (\( P = .004 \)) and the subsequent development of severe aGVHD. Matching by either serology (\( P = .49 \)), IEF (\( P = .12 \)), or RFLP (\( P = .29 \)) was less helpful in predicting severe aGVHD. Analysis by proportional hazards showed a sig-
significant association of low frequency CTLp (RR = 0.28; P = .0035) with a reduced incidence of severe aGVHD (Table 4; Fig 1). The probability of extensive chronic GVHD at 1 year was 38% (95% CI, 27% to 48%). A trend toward an increased incidence of chronic GVHD was seen with HLA class II mismatches detectable by RFLP (P = .13) and female-to-male donor-recipient pairings (P = .11).

Relapse. Thirteen of 115 recipients had a hematologic relapse. The probability of relapse at 3 years was 23% (95% CI, 13% to 36%). Disease phase at transplantation was the only variable found to be associated with relapse, with chronic phase giving a lower risk (RR = 0.71; P = .01; Table 4). No other variable was found to be significant. Of the nine patients who received donor leukocyte infusions, five have responded to therapy (one with hematologic relapse, three with cytogenetic disease only, and one with molecular disease only), one patient cannot be evaluated yet, and three patients have not responded.

TRM. TRM was defined as death in continuing complete remission. Univariate analysis showed that a low CTLp frequency (P = .04), male recipients (P = .05), male donors (P = .05), recipient CMV seronegativity (P = .05), and donor-recipient matching by IEF (P = .005) were all factors associated with decreased TRM. Proportional hazards regression analysis showed that matching by IEF was independently associated with a lower risk of TRM (RR = 0.26; P = .0055). The majority of deaths could be attributed to either infection (24 of 56 cases) or GVHD (16 of 56 cases). There was no significant difference in the TRM when patients with advanced disease were transplanted compared with those transplanted in first chronic phase (43% v 47%).

Survival and LFS. Fifty-seven patients remain alive, 50 without hematologic evidence of leukemia. The overall probabilities of survival and LFS at 3 years were 46% (95% CI, 36% to 56%) and 37% (95% CI, 28% to 47%), respectively. Survival and LFS for the CP and AP groups were 52% and 41%, and 28% and 26%, respectively. The Kaplan-Meier plot of LFS for CP versus AP patients is shown in Fig 2. Univariate analysis revealed improved LFS to be associated not only with first chronic phase (P = .006) but also with male donors (P = .012) and donor-recipient matching by IEF (P = .04). Proportional hazards model analysis indicated that transplantation in first chronic phase, from an IEF-matched donor and with recipient CMV seronegativity, would result in improved survival, and that transplantation in first chronic phase and a combination of recipient CMV seronegativity and an IEF-matched donor were associated with improved LFS (Table 4). There was a trend toward improved survival at 3 years for recipients aged less than 40 years (n

![Fig 1. Probability of developing severe aGVHD according to CTLp frequency (n = 101). Forty-one patients with a high CTLp frequency had a significantly higher probability of developing severe aGVHD when compared with 60 patients with a low CTLp frequency (37% [95% CI, 24% to 52%] v 12% [95% CI, 6% to 22%], P = .004).](www.bloodjournal.org)
matched donors (n = 40) had a 3-year probability of survival and LFS of 73% and 49%, respectively (Fig 3).

**DISCUSSION**

Results from recently published multicenter trials have shown that the ability of interferon-alpha to induce karyotypic remissions in some patients with chronic phase CML may be associated with delayed disease progression and prolonged overall survival compared with conventional chemotherapy. Furthermore, survival benefit may be achieved in the absence of significant cytogenetic responses. For the majority of patients with CML, however, the only hope of cure lies in receiving an allogeneic BMT. Using HLA-identical siblings as donors, several centers showed LFS of the order of 60% or greater, and many workers would consider such a procedure
as first-line therapy. Unfortunately, approximately two-thirds of eligible patients lack a matched sibling donor. The use of phenotypically matched VUDs must, therefore, be considered.

The increased morbidity and mortality associated with VUD BMT for the treatment of CML compared with sibling BMT is well recognized. However, at the present time, the optimal criteria for donor selection are still unclear, as is the best form of GVHD prophylaxis.

The 16% graft failure rate in this series is comparable with that seen by other investigators. However, we have been able to demonstrate an apparent association between graft failure and increasing donor age (Table 3) that previously has not been recognized. The cause of this association is unclear. In animal studies, there is disagreement as to whether there is a reduction in engraftment capability with increasing age. Whether these studies can be extrapolated to humans is unknown. Moreover, if an age-related diminution in engrafting potential exists, it may only manifest itself in the immunologically disparate milieu of phenotypically matched VUD BMT and not genotypically identical sibling BMT. We have not been able to demonstrate a significant association between the type of GVHD prophylaxis (IN-TCD v EX-TCD) or the presence of donor-recipient mismatching and graft failure. Despite the use of cryopreserved chronic phase material to rescue patients with graft failure, graft failure remains a grave complication.

We have found a significant association between relapse rate and disease stage. First chronic phase patients had a probability of relapse at 3 years of 20% (95% CI, 9% to 36%) compared with that in advanced phase patients of 36% (95% CI, 18% to 60%). This raises the possibility of whether the latter group of patients should receive less aggressive GVHD prophylaxis, thus allowing greater expression of the putative graft-versus-leukemia effect of allogeneic BMT with a concomitant reduction in rate of relapse. Such an approach would be safer if the donor were well-matched. The potentially curative management of patients who relapse after BMT is with second BMT or donor leukocyte transfusion (DLT). In sibling BMT, the 2-year probability of LFS is similar for DLT and second BMT: 55% versus 50%, respectively. However, use of DLT before the development of hematologic relapse may improve these results.

Acute GVHD is one of the major causes of morbidity and mortality after VUD BMT, with grade III to IV aGVHD occurring in approximately 50% of patients who receive unmanipulated marrow. In our study population, the proportion of patients developing severe aGVHD was only 24%. This probably reflects the use of some form of T-cell depletion in the majority of patients and is consistent with the findings published by the International Marrow Unrelated Search and Transplant (IMUST) Study group. Proportional hazards model analysis showed that the best predictor of avoiding severe aGVHD was a low pre-BMT CTLp frequency (Fig 1). This is consistent with our recently reported findings in a group of patients with first chronic phase CML and suggests a possible role in the development of aGVHD for HLA disparities not well recognized by the typing methods we have used. It is worth noting that the ability of the CTLp assay to predict severe aGVHD probably depends on the form of GVHD prophylaxis used; with the increased rate of severe aGVHD seen in transplantation with unmanipulated marrow, the CTLp assay has not consistently been found to be a useful predictor. Furthermore, the greater predictive ability of the CTLp assay in this series, compared with both IEF and RFLP, raises the possibility that certain detectable mismatches associated with low CTLp frequencies may not be associated with an increased risk of severe aGVHD and, thus, could be regarded as permissible mismatches when using T-cell depletion.

The overall LFS for this group is comparable with results reported by McGlave et al on behalf of the National Marrow Donor Program and also by the Seattle group. We have confirmed the impact of disease stage, with increased relapse resulting in poorer LFS in patients with advanced disease. IEF mismatch on univariate analysis and in combination with recipient CMV seropositivity in a multivariate analysis was associated with significantly poorer LFS but not relapse, consistent with the unfavorable impact of IEF mismatch on TRM. In only 4 of the 15 donor-recipient pairs with an IEF-detectable mismatch was the discrepancy detected by serologic testing, highlighting the inadequacy of serology for class I HLA-typing for decisions regarding optimal donor selection. Only a small minority of this series of patients underwent BMT within 12 months of diagnosis, so we could not determine if BMT within 12 months of diagnosis would have improved LFS in our patient group, as has been found by other investigators. However, the trend toward increased survival in patients transplanted within 2 years of diagnosis is consistent with this notion.

No difference in LFS was seen between the patients who underwent IN-TCD and those who were transplanted with marrow that had been ex vivo T cell-depleted. The advantage of a reduced incidence of aGVHD with EX-TCD may have been counterbalanced by more prolonged immune dysregulation and, thus, increased susceptibility to infectious complications. Similarly, McGlave et al were unable to show any difference in LFS between EX-TCD and the use of CsA/MTX alone for aGVHD prophylaxis. A multicenter trial under the auspices of the European Group for Blood and Marrow Transplantation has recently commenced comparing CsA/MTX with IN-TCD using Campath 1H in an effort to clarify the optimal form of aGVHD prophylaxis.

Using our present conditioning and aGVHD prophylaxis regimens, performing BMT in patients aged less than 40 years during chronic phase from a donor matched by IEF ensures the greatest likelihood of long-term cure, with such patients having a 3-year probability of survival of 73% and LFS of 50%. Only a small proportion of this series of patients received ganciclovir prophylaxis against CMV reactivation, and whether such an approach will improve outcome in CMV-seropositive recipients compared with historical controls is uncertain. However, it is not unreasonable to assume that with improvements in the prophylaxis, diagnosis, and treatment of CMV, future analyses may no longer indicate CMV seropositivity impacting so unfavorably on survival in VUD BMT.

We have demonstrated that VUD BMT can cure a significant number of appropriately selected patients with CML.
The use of high-resolution tissue typing techniques, including the CTLp assay, allows more accurate outcome prediction after VUD BMT. Thus, the use of these or similarly discriminating methods should enable the more appropriate use of VUD BMT in the management of CML. In young, chronic phase patients lacking an HLA-matched sibling, a search for an unrelated donor should be commenced immediately. If a potential donor, matched using high-resolution typing techniques, is identified, BMT should be considered as soon as possible.

REFERENCES


13. Bidwell JL, Bidwell EA, Savage DA, Middleton D, Klouda PT, Bradley BA: A DNA-RFLP typing system that positively identifies serologically well defined and ill defined HLA-DR and DQ alleles, including DRw10. Transplantation 45:640, 1988


28. Allan NC, Richards SM, Shepherd PCA: UK Medical Re-


Bone marrow transplantation for chronic myeloid leukemia with volunteer unrelated donors using ex vivo or in vivo T-cell depletion: major prognostic impact of HLA class I identity between donor and recipient

A Spencer, RM Szydlo, PA Brookes, E Kaminski, S Rule, F van Rhee, KN Ward, G Hale, H Waldmann and JM Hows