Survival in first or second remission after lymphocyte-depleted transplantation for Philadelphia chromosome-positive CML in first chronic phase

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Summary:

We studied the outcome of BMT in 38 consecutive CML patients in CP1 who received transplants depleted of lymphocytes using counterflow centrifugation. In all patients the conditioning regimen was intensified by the addition of anthracyclines. Donors were HLA, MLC-identical siblings. Six patients (16%) died within 6 months. All 37 patients with a follow-up of more than 0.5 months engrafted and only one (3%) suffered from acute GVHD ≥ grade 3. Chronic GVHD was evaluable in 33 patients and was extensive in six (18%). The projected 5-year probabilities of hematologic, cytogenetic and molecular relapse were 30% (95% confidence interval (CI), 10–49%), 35% (95% CI, 14–56%), and 34% (95% CI, 13–55%), respectively. The projected 5-year probability of survival was 68% (95% CI, 50–86%). Projected at 5 years, probabilities of leukemia-free survival (LFS) in hematologic, cytogenetic and molecular remission were 55% (95% CI, 37–73%), 51% (95% CI, 32–69%), and 51% (95% CI, 32–70%), respectively. All patients with relapse but one who relapsed in blastic phase were treated with retransplantation (n = 1) or with the infusion of lymphocytes (n = 6). Six patients regained second hematologic remission and five entered second cytogenetic and molecular remission. Including these patients, the probability of survival in first or second hematologic remission at the end of follow-up was 68% (95% CI, 50–86%). The probabilities of survival in first or second cytogenetic and molecular remission at the end of follow-up were both 61% (95% CI, 42–80%). We advocate revaluation of T cell depletion of donor marrow for patients who are not treated with «-interferon. The clinical relevance of a small number of persisting cells expressing the BCR-ABL mRNA has been discussed by several authors. It has been speculated whether the complete disappearance of the BCR-ABL gene rearrangement was necessary for cure of the disease. However, using PCR with nested primers, a relationship was observed between PCR positivity and relapse. Quantitative PCR in consecutive samples showed that the number of residual tumor cells had important prognostic value.

For CML patients who relapse, treatment options are limited. In patients with cytogenetic or hematological relapse, a interferon significantly improved the 2-year probability of survival but long-term survival did not differ from that in patients who were not treated with a interferon. Retransplantation is associated with a high treatment-related morbidity and mortality. Following a second transplant for CML 2-year probabilities (95% confidence interval (CI)) of treatment-related mortality, relapse and leukemia-free survival were 35% (95% CI, 20–53%), 43% (95% CI, 27–62%), and 37% (95% CI, 23–53%), respectively. Retransplantation may become more successful since transplant-related mortality can be reduced by less intensive conditioning regimens, such as high-dose busulphan alone. Recently, several authors have reported on the treatment of relapse after bone marrow transplantation with the infusion of leukocytes from the original marrow donor. Complete remission was induced in 54 patients (73%) with relapsed CML.

In the present analysis we report on the outcome of transplantation for CML-CP1 with HLA, MLC-identical sibling grafts depleted of lymphocytes using counterflow centrifug-
ation. All patients received a conditioning regimen which was intensified by the addition of anthracyclines. In all patients with a relapse, except one who relapsed into blastic phase, we attempted to induce second remission with retransplantation (n = 1) or with the infusion of lymphocytes from the original marrow donor (n = 6). Using this form of immunotherapy, we observed high probabilities of LFS and of survival in first or second remission.

Materials and methods

Patients, donors and methods

Between July 1986 and August 1995, 61 consecutive patients were transplanted for CML-CP1. Fifteen patients were excluded from analysis because they received marrow from non-HLA, MLC-identical family donors (n = 7) or matched unrelated donors (n = 8). Another eight patients were excluded since indication for transplantation was Ph chromosome and BCR/ABL breakpoint negative CML (n = 1) or conditioning for BMT was performed without the addition of anthracyclines (n = 7). Median age of the remaining 38 recipients (18 females and 20 males) was 37 (range 19–54) years. The donors (14 females and 24 males) were aged from 17 to 71 (median 39) years.

Conditioning

Thirty-four patients were conditioned with cyclophosphamide 60 mg/kg body weight/day on days –6 and –5 and total body irradiation (TBI) given in two equal fractions of 4.5 Gy each on days –2 and –1 using a 16 MV or 18 MV photon beam linear accelerator. The conditioning regimen was intensified by the addition of anthracyclines (daunorubicin to a total dose of 156 mg/m² or idarubicin to a total dose of 42 mg/m²) by continuous intravenous (i.v.) infusion for 2 or 7 days. Four patients were conditioned without TBI. They received idarubicin to a total dose of 42 mg/m² by continuous i.v. infusion on days –12 and –11, busulphan 4 mg/kg/body weight (days –8 to –5 inclusive) and cyclophosphamide 60 mg/kg/body weight on days –3 and –2. Bone marrow was infused on day 0.

Donor marrow

Donor marrow was depleted of lymphocytes by density gradient centrifugation followed by counterflow centrifugation as described before. In summary: bone marrow is filtered through a nylon filter with a pore size of 70 μm. After removal of plasma and fat, a mononuclear cell fraction with a density <1.073 g/ml is isolated in gradients of Percoll (Percoll, Uppsala, Sweden). Then the low density mononuclear fraction is pumped into the separation chamber which is placed eccentrically in a centrifugation rotor. The separation capacity of the original single-chamber rotor (Beckman JE-6; Beckman, Palo Alto, CA, USA) has been enhanced by the development of a multi-chamber counterflow centrifugation system with four separation chambers reducing the total time of the elutriation procedure to about 3 h. The transparent chambers (International Medical, Zutphen, The Netherlands) have been placed eccentrically in a rotor (type Mark-1; Dijkstra Vereenigde, Amsterdam, The Netherlands) that has been installed in a modification (Curam-3000) of the Varifuge-RF centrifuge (Heraeus Separationstechnik, Ostenrode, Germany). The particles in the separation chamber are subjected to two opposing forces: the centrifugal force depending upon the rotor speed and the centripetal force caused by the continuous fluid stream pumped into the chamber with a direction towards the rotor axis. At equilibrium the cells are positioned according to their size with the larger cells in the more centrifugal position and the smaller cells in the more centripetal direction. By increasing the centripetal force and/or decreasing the centrifugal force this equilibrium shifts towards the outlet of the chamber and the smaller cells are the first to leave the chamber. The rotor outlets are monitored for cell number and size using a computerized cell scatter-controlled device. The cells are collected in several rotor speed fractions. In each fraction the number of nucleated cells is measured by using a cell counter (Coulter Counter; Coulter Corporation, Hialeah, FL, USA). After binding to CD3-FITC monoclonal antibody (Dakopatts, Glostrup, Denmark) the percentage of T lymphocytes is determined by using an Epics Elite flow cytometer (Coulter Corporation). With this technique it is permitted to add a fixed number of T cells to each graft individually.

The marrow that was ultimately given to the recipient consisted of the larger cell fractions with an absolute total number of T lymphocytes between 0.4 and 3.2 (median 1.1) x 10⁶/kg body weight of the recipient.

Immunoprophylaxis after transplantation

Immunoprophylaxis post-transplant consisted of cyclosporine A (CsA) alone, 3 mg/kg body weight/day by continuous i.v. infusion from days –1 to +14, followed by 2 mg/kg body weight/day. As soon as CsA could be taken orally, it was given in a dose of 6 mg/kg body weight/day to 12 weeks after BMT, followed by a gradual tapering off and discontinuation after 16 weeks post-grafting.

Management of patients

All patients were managed in single rooms with filtered air under positive pressure throughout the transplantation period inside the hospital, and all received oral selective gut decontamination, as well as co-trimoxazole for Pneumocystis carinii prophylaxis and oral acyclovir for prophylaxis of herpes infection.

GVHD

The clinical manifestations of acute GVHD were graded 1 to 4 according to the criteria described by Glucksberg et al. Chronic GVHD was classified as limited or extensive as described by Shulman et al. If GVHD occurred ≤3 months after retransplantation or ≤3 months after the infusion of lymphocytes it was defined as acute. If the patients had GVHD beyond 3 months, it was defined as chronic.
Bone marrow samples

As part of an ongoing study on chimerism, bone marrow samples were taken before BMT, 0.5 and 1 year after transplantation and annually thereafter. From 1991 onwards, bone marrow samples were analyzed prospectively for the presence of BCR/ABL breakpoint molecules. PCR analysis of samples taken before 1991 was done retrospectively in liquid nitrogen stored bone marrow. If cytogenetic analysis for the Ph chromosome and/or PCR for the BCR/ABL breakpoint was positive, bone marrow samples were taken and analyzed at shorter intervals.

Cytogenetic analysis

For demonstration of the Ph chromosome, bone marrow cells were prepared directly and/or cultured for 24 h in RPMI 1640 medium (Gibco, Paisley, UK) without mitogens and processed for GTG banding. As a standard, 32 metaphases were analyzed.

PCR analysis

Two series of PCR were performed as described before. In summary: RNA was isolated from bone marrow cells in a modification of the method of Chomczynski and Sacchi. Primers for the synthesis of cDNA (A21) and for the two series of PCR were derived from the Sequence, EMBL/Genebank accession No. M30828 (Heidelberg, Germany). Primers B11 and A21 were used in the first 30-cycle series and the internal primers B12 and A22 in the second 35-cycle PCR. The quality of each RNA sample was assessed in a reverse transcriptase reaction followed by PCR on β₂-microglobulin RNA. Strict precautions were taken to avoid contaminations. In each PCR, both positive and negative controls were used. PCR products were analyzed by hybridization after Southern blotting using a 32P-labeled c-ABL probe recognizing the 5' part of exon 2 of the c-ABL gene. With this PCR, we were able to demonstrate one K562 cell among 10⁷ normal cells.

Definitions

Hematologic relapse was defined as the reappearance of clinical features and laboratory findings characteristic for CML. Cytogenetic relapse was the recurrence of metaphases with the Ph chromosome. A molecular relapse was defined as a positive PCR at two or more consecutive points of time after BMT.

If PCR for BCR/ABL was negative and the patient had no cytogenetic or hematologic relapse, he was in molecular remission. The patient was in cytogenetic remission if the bone marrow showed no Ph chromosome-positive metaphases and the patient had no hematologic relapse. Hematologic remission was defined as the disappearance of signs or symptoms of CML and normalization of blood counts and bone marrow cellularity in the absence of antileukemic therapy.

LFS was defined as survival in first hematologic remission. If LFS refers to survival in first cytogenetic or first molecular remission this is stated in the text. Patients survived in second hematologic, cytogenetic or molecular remission if they attained hematologic, cytogenetic or molecular remission, respectively, and remained in that remission until the end of follow-up. For these patients follow-up was calculated from T cell-depleted BMT onwards.

Take failure was defined as primary (leukocytes always <1.0 × 10⁹/l) or as secondary (leukocytes ≥1.0 × 10⁹/l on 3 or more consecutive days with disappearance of leukocytes in further follow-up).

Follow-up

Follow-up was until death or 1 August 1996. For surviving patients, follow-up was at least 12 months.

Treatment of relapse

Before the era of lymphocyte infusions for the treatment of relapse, one patient had been retransplanted directly with unmanipulated marrow from the original donor. Conditioning for second BMT consisted of busulphan 16 mg/kg and cyclophosphamide (200 mg/kg). Six patients with a relapse were given lymphocytes from the original marrow donor as described before.

Statistics

Fisher's exact test was used for comparison of two proportions. Calculations were made using the Statistica Release 4.1 programme (Apple Computer, Cupertino, CA, USA). \( P \) values <0.05 were considered significant. The probabilities of relapse, survival and LFS were calculated using the Kaplan-Meier method. Differences in probabilities were calculated using the log-rank test and Wilcoxon’s test. Calculations were made using the SAS programme (SAS Institute, Cary, NC, USA). \( P \) values <0.05 were considered significant. The Kaplan-Meier method was also used for the calculation of the probability of survival in first or second remission at the end of follow-up. For this calculation, patients who relapsed and attained second remission after retransplantation or after the infusion of lymphocytes were not scored for relapse. Probability of survival in first or second remission was calculated from T cell-depleted BMT onwards and was given only for the endpoint of follow-up. Probability of survival in second remission was given separately for survival in second hematologic, cytogenetic and molecular remission.

Results

Interval from diagnosis to transplantation

Interval from diagnosis to transplantation varied from 3 to 62 (median 10) months. Twenty-nine patients (76%) were transplanted ≤12 months after diagnosis of CML. Four of 29 patients (14%) who were transplanted ≤1 year after diagnosis relapsed hematologically compared to three of nine (33%) transplanted beyond 1 year (\( P >0.05 \)). A comparable difference was observed for cytogenetic and molecular relapse, respectively (data not shown).
Take failure

All 37 patients with a follow-up of more than 0.5 months engrafted. Two patients (5%) suffered from secondary take failure at 2 and 3 months, respectively. Both were retransplanted with an unmanipulated graft after conditioning with total lymphoid irradiation given in two fractions of 2 Gy on each of 3 consecutive days to a total dose of 12 Gy. One of these patients died from GVHD at 3 months after retransplantation. The other remained in molecular remission at 40 months after the first BMT and 38 months after retransplantation.

GVHD

Acute GVHD occurred after T cell-depleted BMT and before retransplantation or the infusion of lymphocytes in 20 of 37 engrafted patients (54%). Twelve patients (32%) had acute GVHD grade 1, seven (19%) grade 2 and one patient (3%) suffered from grade 3 acute GVHD.

Chronic GVHD before retransplantation or before lymphocyte infusion occurred in 16 of 33 patients (48%) with a follow-up of >3 months. GVHD was limited in 10 (30%) and extensive in six patients (18%).

The incidence but not the severity of acute GVHD in the nine patients after retransplantation (n = 3) or after the infusion of lymphocytes (n = 6) was significantly higher than that observed in these patients after T cell-depleted BMT (Table 1). The incidence of extensive chronic GVHD was significantly higher after retransplantation (n = 1) or after the infusion of lymphocytes (n = 6) in the seven patients who were evaluable for chronic GVHD after T cell-depleted BMT and after retransplantation or after lymphocyte infusion and this is shown in Table 1.

Mortality

Ten of 38 patients (26%) died at 0.5–69 (median 6) months after lymphocyte-depleted BMT. The principal causes of death and the interval between T cell-depleted BMT and death are given in Table 2. Only one patient died from relapse at 50 months after T cell-depleted BMT. He relapsed at 48 months in blastic phase and was treated symptomatically only. One patient died in molecular remission from overwhelming pneumococcal sepsis at 69 months after the first BMT and 27 months after retransplantation for relapse. She was not immunized with pneumococcal vaccine after (re)transplantation. Cause of death was Epstein-Barr virus-associated secondary lymphoma in one patient. She died 17 months after T cell-depleted transplantation.

Relapse

Hematologic relapse was observed in seven patients at 12–48 (median 24) months after T cell-depleted BMT. In three patients hematologic relapse occurred at 4–12 (median 6) months after cytogenetic and at 6–23 (median 12) months after molecular relapse. The probability of hematological relapse was 30% (95% CI, 10–49%) and is given in Figure 1.

Eight patients had a cytogenetic relapse at 6–48 (median 24) months after BMT. In one of these patients cytogenetic relapse was preceded by molecular relapse which occurred 19 months earlier. The probability of cytogenetic relapse was 35% (95% CI, 14–56%) and is shown in Figure 1.

Thirteen patients had positive PCR for the BCR/ABL breakpoint at 6–48 (median 24) months after transplantation. In five patients PCR was only positive at one timepoint after BMT. At that time, none of these five recipients had cytogenetic or hematologic relapse and PCR was negative in two to five consecutive analyses in later follow-up. As given in the definitions, these patients were not considered as having a molecular relapse. Molecular relapse occurred in eight patients at 6–48 (median 18) months after transplantation. One patient had a molecular relapse without evidence of cytogenetic or hematologic relapse at the time of molecular relapse. Nineteen and 23 months later he relapsed cytogenetically and hematologically, respectively.

Table 1  The incidence and severity of acute and chronic GVHD after T cell-depleted BMT and after retransplantation or the infusion of lymphocytes

<table>
<thead>
<tr>
<th></th>
<th>After T cell-depleted BMT</th>
<th>After retransplantation or lymphocyte infusion</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute GVHD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>none</td>
<td>6 (67)</td>
<td>1 (11)</td>
<td>0.025</td>
</tr>
<tr>
<td>grade 1</td>
<td>2 (22)</td>
<td>3 (33)</td>
<td>NS</td>
</tr>
<tr>
<td>≥ grade 2</td>
<td>1 (11)</td>
<td>5 (56)</td>
<td>NS</td>
</tr>
<tr>
<td>≥ grade 3</td>
<td>0 (0)</td>
<td>2 (22)</td>
<td>NS</td>
</tr>
<tr>
<td>Chronic GVHD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>none</td>
<td>5 (71)</td>
<td>1 (14)</td>
<td>NS</td>
</tr>
<tr>
<td>limited</td>
<td>2 (29)</td>
<td>2 (29)</td>
<td>NS</td>
</tr>
<tr>
<td>extensive</td>
<td>0 (0)</td>
<td>4 (57)</td>
<td>0.035</td>
</tr>
</tbody>
</table>

NS = not significant.
Table 2  Principal causes of death and interval between T cell-depleted BMT and death

<table>
<thead>
<tr>
<th>Principal cause of death</th>
<th>n</th>
<th>Interval BMT and death (in months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspergillosis</td>
<td>2</td>
<td>1.5 and 2</td>
</tr>
<tr>
<td>Cytomegalovirus-associated interstitial pneumonitis</td>
<td>2</td>
<td>6 and 6</td>
</tr>
<tr>
<td>Adult respiratory distress syndrome</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td>Graft-versus-host disease (n = 2) after T cell-depleted BMT</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>after retransplantation with an unmanipulated graft</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td>Secondary lymphoma</td>
<td>1</td>
<td>50</td>
</tr>
<tr>
<td>Untreated relapse</td>
<td>1</td>
<td>69</td>
</tr>
<tr>
<td>Pneumococcal sepsis after retransplantation</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Figure 1  Probability of hematologic (a) and cytogenetic (b) relapse. Tick marks in the relapse curves denote survivors in first hematologic (a) and first cytogenetic (b) remission after T cell-depleted BMT. The difference between the probabilities of relapse was not significant.

The probability of molecular relapse was 34% (95% CI, 13–55%). Since the curve for the probability of molecular relapse is almost identical to that of cytogenetic relapse, this curve is not given.

Survival

Twenty-eight of 38 patients (74%) were alive at 12–120 (median 51) months after BMT. Of these, seven were alive after retransplantation for secondary take failure (n = 1) or after the infusion of lymphocytes (n = 6). The probability of survival is given in Figure 2 and was 68% (95% CI, 50–86%).

Probability of leukemia-free survival

At 12–120 (median 43) months, 23 of 38 patients (61%) were alive and in first hematologic remission. Probability of LFS was 55% (95% CI, 37–73%) and is given in Figure 3. Twenty-two of 38 patients (58%) were alive and in first cytogenetic remission at 12–120 (median 43) months after T cell-depleted BMT. The probability of LFS in first cytogenetic remission was 51% (95% CI, 32–69%) and is given in Figure 3. At 12–120 (median 43) months, 22 of 38 patients (58%) were alive and in first molecular remission. Probability of LFS in molecular remission was 51% (95% CI, 32–70%). Since the curve for LFS in molecular...
remission was almost identical to that of LFS in cytogenetic remission this curve is not shown.

**Probability of survival in first or second remission**

Seven patients were treated for relapse with retransplantation \((n = 1)\) or with the infusion of lymphocytes from the original marrow donor \((n = 6)\). The patient who was given a second transplant with an unmanipulated graft for hematologic relapse died in molecular remission from overwhelming pneumococcal sepsis at 27 months after retransplantation. Five patients were given lymphocytes for hematologic relapse and all regained hematologic remission and all but one entered second cytogenetic and molecular remission. One patient received lymphocytes for cytogenetic relapse only. At 12–120 (median 51) months after T cell-depleted BMT, 28 of 38 patients (74\%) were alive and in first or second hematologic remission. The probability of survival in first or second hematologic remission at the end of follow-up and calculated from T cell-depleted BMT onwards was 68\% (95\% CI, 50–86\%). Twenty-six of 38 patients (68\%) were alive and in cytogenetic remission at 12–120 (median 51) months after lymphocyte-depleted BMT. The probability of survival in first or second cytogenetic remission at the end of follow-up from T cell-depleted BMT onwards was 61\% (95\% CI, 42–80\%). This probability will increase further if the two patients who still had Ph chromosome-positive metaphases after lymphocyte infusion enter a second cytogenetic remission. The probability of survival in first or second molecular remission at the end of follow-up and calculated from T cell-depleted BMT onwards was identical to that of survival in first or second cytogenetic remission.

**Discussion**

In the present analysis, acute GVHD \(\geq\) grade 2 occurred in eight of 37 patients (22\%). This contrasts favorably with results from the study for the EBMT of Ringdén et al. They observed acute GVHD \(\geq\) grade 2 in 305 of 780 patients (39\%) after first transplantation for CML-CP1 with grafts from HLA-identical siblings. In 22\% of cases, grafts were T cell-depleted. In the same study, day 100 mortality for CML-CP1 patients was 17\%. In contrast, only three of 38 patients (8\%) in the present analysis had died at day 100. The relatively low incidence of acute GVHD \(\geq\) grade 2 in our patients is reflected in the relatively low day 100 mortality. Seven out of 10 patients died beyond day 100. Nowadays, three of these patients might have been saved by adoptive immunotherapy,\(^{47}\) an earlier diagnosis of impending relapse\(^{28}\) or by pneumococcal prophylaxis.\(^{38}\)

The incidence of acute and the severity of chronic GVHD after retransplantation with an unmanipulated graft or after the infusion of lymphocytes was higher than that observed in these patients after T cell-depleted BMT. This observation shows that for each patient individually the occurrence and severity of GVHD depends on the number of lymphocytes infused.

The projected 5-year probability of hematologic relapse in our patients was 30\%. The probability of hematologic relapse after transplantation for CML-CP1 with T cell-depleted grafts varied from 48\% at 3 years after BMT to 65\% at 8 years post-transplant.\(^{5,41}\) Several factors may have contributed to this relatively low incidence of hematologic relapse observed in our population. Firstly, when counterflow centrifugation is used for depletion of lymphocytes, it retains the relatively large lymphocytes in the graft. This lymphocyte population contains most of the large granular lymphocytes which belong to the MHC nonrestricted cytotoxic cells (natural killer (NK) cells) having the capability to inhibit the growth of fresh clonogenic CML cells.\(^{29}\) This suggests that NK cells are precursors or effectors of graft-versus-leukemia reaction. The phenotype of MHC nonrestricted cytotoxic cells is predominantly CD3\(^+\) and CD16\(^+\) while a small population bears CD3 and CD16.\(^{20}\) These cells will also be eliminated if depletion techniques with monoclonal antibodies are used for lymphocyte depletion. Secondly, the addition of anthracyclines to the conditioning regimen may have decreased the probability of relapse as described earlier.\(^{36–38}\) However, this appeared not to be due to the additional elimination of leukemic cells by anthracyclines but more to the relatively higher incidence of GVHD as reported by Bär et al.\(^{51}\) They observed a lower incidence of mixed erythrocyte chimerism, a higher incidence of acute GVHD \(\geq\) grade 1 and a trend to a lower relapse rate in patients conditioned with the addition of anthracyclines. The addition of anthracyclines to the conditioning regimen may have resulted primarily in an additional elimination of immunocompetent autologous T lymphocytes tipping the immunological balance towards the low number of T lymphocytes given within the graft. Thirdly, 29 of 38 patients (76\%) were transplanted within 1 year after diagnosis of CML and this may be another explanation for the relatively low probability of relapse. Patients transplanted beyond 1 year after diagnosis of CML tended to have a higher relapse incidence than patients transplanted within 1 year after diagnosis, although these differences were not significant.\(^{2–4}\)

The probabilities of cytogenetic and molecular relapse did not differ significantly from that of hematologic relapse. In all but one of the eight patients cytogenetic relapse heralded or occurred simultaneously with hematologic relapse and molecular relapse preceded or coincided with cytogenetic relapse. Our policy is to treat patients only with the infusion of donor lymphocytes if cytogenetic relapse has occurred not to be due to the additional elimination of leukemic cells by anthracyclines but more to the relatively higher incidence of GVHD as reported by Bär et al.\(^{51}\) They observed a lower incidence of mixed erythrocyte chimerism, a higher incidence of acute GVHD \(\geq\) grade 1 and a trend to a lower relapse rate in patients conditioned with the addition of anthracyclines. The addition of anthracyclines to the conditioning regimen may have resulted primarily in an additional elimination of immunocompetent autologous T lymphocytes tipping the immunological balance towards the low number of T lymphocytes given within the graft. Thirdly, 29 of 38 patients (76\%) were transplanted within 1 year after diagnosis of CML and this may be another explanation for the relatively low probability of relapse. Patients transplanted beyond 1 year after diagnosis of CML tended to have a higher relapse incidence than patients transplanted within 1 year after diagnosis, although these differences were not significant.\(^{2–4}\)

LFS after transplantation for CML-CP1 with T cell-depleted and HLA-identical sibling grafts varied from 37\% at 2 years to 21\% at 8 years after BMT.\(^{47,52}\) After transplantation for CML-CP1 with unmanipulated grafts from HLA-identical siblings, LFS varied from 44\% at 8 years to 68\% at 2 years post-transplant.\(^{5,41}\) In contrast to Goldman et al\(^{2}\) others concluded that T cell-depletion significantly reduced LFS.\(^{3,4}\) In the present analysis LFS projected at 5 years after BMT was 55\% and is comparable with LFS obtained in recipients of unmanipulated marrow.

Patients who relapse from CML can be treated with retransplantation or preferably with the infusion of lympho-
lymphocyte-depleted transplantation for CML-CP1
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cytes from the original bone marrow donor. Including these patients we found a high probability of survival in first or second remission at the end of follow-up.

We have demonstrated that LFS after BMT for CML-CP1 with grafts from HLA-identical sibling donors which are depleted of lymphocytes using counterflow centrifugation is comparable with that obtained in recipients of unmanipulated grafts. Even after intensification of the conditioning regimen by the addition of anthracyclines, probability of relapse remains higher than in recipients of unmanipulated grafts. However, the major advantage of T-cell-depleted transplantation remains the lower mortality caused by GVHD. The higher probability of relapse is not the major drawback anymore since patients with a relapse can be offered a very good chance of attaining second remission by infusion of lymphocytes from the original donor. For that reason we advocate a revaluation of T-cell-depletion of donor marrow for patients with CML-CP1 at high risk of developing GVHD.

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