Allogeneic Hematopoietic Cell Transplantation After Nonmyeloablative Conditioning

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INTRODUCTION

High-dose chemo- or chemoradiotherapy followed by allogeneic hematopoietic cell transplantation (HCT) has been recognized as an effective therapy for a number of hematologic malignancies with tumor cells resistant to conventional doses of chemotherapy (1). The aims of the high-dose conditioning are (i) to abolish host immune responsiveness prior to transplantation to avoid graft rejection and (ii) to deliver doses of cytotoxic anticancer agents beyond the range that is toxic to the bone marrow cells, thereby potentially increasing antitumor efficacy (1). The curative potential of allogeneic HCT is not only due to the high-dose chemoradiotherapy but also due to immune-mediated graft-versus-tumor (GVT) effects (2–4).

The existence of a GVT effect was first suggested by Barnes et al. in 1956 (5). They observed that mice receiving syngeneic HCT and injection of congenic leukemic cells after total body irradiation (TBI) almost uniformly died from
leukemia, whereas a number of mice receiving histoincompatible marrow were cured of leukemia but eventually died from the graft-versus-host disease (GVHD). The authors suggested that a reaction of the donor spleen cells might kill cancer cells. This hypothesis was evinced two decades later in humans by studies reporting reduced leukemic relapse rates in allografted patients who developed GVHD compared with those who did not (2,3). The GVT effect was further demonstrated by other investigators who observed increased risks of relapse in patients given T-cell-depleted grafts and in recipients of syngeneic transplants (3).

Those observations led several groups of investigators to investigate the curative potential of donor lymphocytes infusions (DLI) in patients who had relapsed after allogeneic HCT (4). Two large multicenter studies, one from the European Group for Blood and Marrow Transplantation (EBMT) (4) and the other from North America (6), have analyzed the efficacy of DLI in more than 400 patients (Table 1). DLI induced complete remissions in more than 60% of patients with chronic myeloid leukemia and 10% to 40% of patients with other hematologic malignancies. Typically, achievement of complete remissions required several weeks. For example, an average time of four to six months was required before molecular remission was achieved in patients with relapsed chronic myeloid leukemia (4). While 50% of patients without acute GVHD showed tumor regression, this increased to 75% and 85% in patients with grade I or grades II to IV acute GVHD, respectively (4). Similarly, chronic GVHD was associated with disease responses (4,6). DLI have been given without any other treatment in patients with indolent disease such as chronic myeloid leukemia in chronic phase, while chemotherapy has been given before DLI in a number of

Table 1  Results of Donor Lymphocyte Infusions as Treatment of Relapse After HLA-Matched HCT Following Myeloablative Conditioning

<table>
<thead>
<tr>
<th>Condition</th>
<th>North America (6)</th>
<th>EBMT (4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Complete response/</td>
<td>Complete response/</td>
</tr>
<tr>
<td></td>
<td>evaluable patients (%)</td>
<td>evaluable patients (%)</td>
</tr>
<tr>
<td>Chronic myeloid leukemia</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cytogenetic/molecular relapse</td>
<td>3/3 (100)</td>
<td>40/50 (80)</td>
</tr>
<tr>
<td>Hematologic relapse</td>
<td>25/34 (74)</td>
<td>88/114 (77)</td>
</tr>
<tr>
<td>Accelerated phase/blast crisis</td>
<td>5/18 (28)</td>
<td>13/36 (36)</td>
</tr>
<tr>
<td>Acute myeloid leukemia/</td>
<td>8/44 (18)</td>
<td>16/59 (27)</td>
</tr>
<tr>
<td>myelodysplastic syndrome/</td>
<td></td>
<td></td>
</tr>
<tr>
<td>polycythemia vera</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Acute lymphoblastic leukemia</td>
<td>2/11 (18)</td>
<td>3/20 (15)</td>
</tr>
<tr>
<td>Multiple myeloma</td>
<td>2/4 (50)</td>
<td>5/17 (29)</td>
</tr>
<tr>
<td>Non-Hodgkin’s lymphoma</td>
<td>0/6 (0)</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviation: EBMT, European Group for Blood and Marrow Transplantation.
patients with more aggressive diseases. Figure 1 shows overall survival in 48 patients given DLI for progressive disease/relapse after nonmyeloablative conditioning (7).

Because of regimen-related toxicities, the use of high-dose myeloablative conditionings has been restricted to younger and medically fit patients. This is unfortunate, given that the median age at diagnosis of patients with acute and chronic myeloid leukemias, chronic lymphocytic leukemia, non-Hodgkin’s lymphomas (NHLs), myelodysplastic syndromes, and multiple myeloma ranges from 65 to 70 years (1). In 1971, Santos et al. reported that conditioning with cyclophosphamide alone, although nonmyeloablative enabled sustained engraftment of transplanted allogeneic hematopoietic cells in patients with advanced leukemia (8). Unfortunately, tumor cells were not completely eradicated, and all patients eventually relapsed. While cyclophosphamide became the conditioning regimen of choice for patients with aplastic anemia (1), it was abandoned as the sole conditioning regimen in patients with hematologic malignancies. In 1974, Graw et al. reported a few cures in patients with acute leukemia given allogeneic marrows after a reduced-intensity (9) conditioning regimen combining BCNU, cytarabine, cyclophosphamide, and thioguanine (10). The growing evidence of the power of GVT effects, as demonstrated by the efficacy of DLI, incited several groups of investigators to develop new reduced-intensity (11–14) or truly nonmyeloablative conditioning regimens (15–17) allowing older patients and those with comorbidities to benefit from GVT effects (Table 2).
### Table 2  Examples of Reduced-Intensity or Nonmyeloablative Conditioning Regimens

<table>
<thead>
<tr>
<th>Center (Ref.)</th>
<th>Preparative regimens</th>
<th>Postgraft immunosuppression</th>
<th>No. of pts (median age in yr)</th>
<th>Diseases</th>
<th>GVHD</th>
<th>NRM (days after transplant)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Reduced-intensity regimens</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MD Anderson (12)</td>
<td>Fludarabine 25 mg/m&lt;sup&gt;2&lt;/sup&gt;/day (or 2-CDA 12 mg/m&lt;sup&gt;2&lt;/sup&gt;) x 5 days, Melphalan 140-180 mg/m&lt;sup&gt;2&lt;/sup&gt;</td>
<td>FK506 + MTX</td>
<td>86 (52)</td>
<td>Hematological malignancies</td>
<td>Acute (grade II-IV)</td>
<td>49%</td>
<td>Chronic</td>
</tr>
<tr>
<td>UK consortium</td>
<td>Fludarabine 30 mg/m&lt;sup&gt;2&lt;/sup&gt;/day x 5 days, Melphalan 140-180 mg/m&lt;sup&gt;2&lt;/sup&gt;, Alemtuzumab 20 mg/day x 5 days</td>
<td>CSP</td>
<td>88 (48)</td>
<td>Non-Hodgkin's lymphoma</td>
<td>Acute (grade II-IV)</td>
<td>15%&lt;sup&gt;c&lt;/sup&gt;</td>
<td>Chronic</td>
</tr>
<tr>
<td>Hadassah-Hebrew University (11)</td>
<td>Fludarabine 30 mg/m&lt;sup&gt;2&lt;/sup&gt;/day x 6 days, Busulfan (p.o.) 4 mg/kg/day x 2 days, ATG 5-10 mg/kg/day x 4 days</td>
<td>CSP +/- MTX</td>
<td>24 (35)</td>
<td>Chronic myeloid leukemia in first chronic phase.</td>
<td>Acute (grade II-IV)</td>
<td>75%&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Chronic</td>
</tr>
<tr>
<td>No. of pts</td>
<td>Median age in yr</td>
<td>Diseases</td>
<td>Acute (grade II–IV)</td>
<td>Chronic</td>
<td>NRM (days after transplant)</td>
<td>Outcome</td>
<td></td>
</tr>
<tr>
<td>------------</td>
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<td></td>
</tr>
<tr>
<td>110 (44)</td>
<td>30% at 1 yr</td>
<td>Hematological malignancies</td>
<td>33%</td>
<td>59%</td>
<td>2 y DFS 40%</td>
<td>80/15 pts survived between 121 and 409 (median: 200) days. 2 yr DFS: 84%</td>
<td></td>
</tr>
<tr>
<td>15 (50)</td>
<td>Hematological + solid malignancies</td>
<td>40%</td>
<td>50%</td>
<td>巨</td>
<td>2 y DFS 59</td>
<td>64%</td>
<td></td>
</tr>
<tr>
<td>20 (51)</td>
<td>Indolent lymphomas</td>
<td>20%</td>
<td>64%</td>
<td>巨</td>
<td>2 y DFS 45</td>
<td>64%</td>
<td></td>
</tr>
<tr>
<td>20 (51)</td>
<td>Indolent lymphomas</td>
<td>20%</td>
<td>64%</td>
<td>巨</td>
<td>2 y DFS 45</td>
<td>64%</td>
<td></td>
</tr>
</tbody>
</table>

### Preparative regimens

**Washington University (14)**

- TBI 5.5 Gy
- Cyclophosphamide 120 mg/kg
- Cyclophosphamide (325 mg/m²/day × 5 days)
- 2 days
- 2 yr DFS 40%

**MD Anderson (15)**

- Fludarabine 25 mg/m²/day × 5 days
- 60 mg/m²/day × 3 days
- 2 days
- 2 yr DFS 40%

**National Institutes of Health (17)**

- Fludarabine 25 mg/m²/day × 5 days
- 60 mg/m²/day × 3 days
- 2 days
- 2 yr DFS 40%

**Nonmyeloablative regimens**

- Fludarabine 25 mg/m²/day × 5 days
- 60 mg/m²/day × 3 days
- 2 days
- 2 yr DFS 40%

### Postgraft immunosuppression

- CSP + MTX + steroids
- CSP + MTX + steroids
- CSP + MTX + Rituximab
- CNS prophylaxis

### Center (Ref.)

- NMDM
- HLA matched
- Genetically matched

### Outcome

- 2 yr DFS: 84%
- 2 yr DFS: 84%
- 2 yr DFS: 84%
- 2 yr DFS: 84%
<table>
<thead>
<tr>
<th>Center (Ref.)</th>
<th>Preparative regimens</th>
<th>Postgraft immunosuppression</th>
<th>No. of pts (median age in yr)</th>
<th>Diseases</th>
<th>Acute (grade II-IV)</th>
<th>Chronic</th>
<th>NRM (days after transplant)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>FHCRC consortium&lt;sup&gt;a&lt;/sup&gt;&lt;br&gt;(24)</td>
<td>TBI 2 Gy +/- Fludarabine 30 mg/m²/day × 3 days.</td>
<td>CSP + MMF</td>
<td>451 (55)</td>
<td>Hematological malignancies.</td>
<td>48%</td>
<td>44%&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7% at 100 days</td>
<td>2-year OS: 51%</td>
</tr>
</tbody>
</table>

<sup>a</sup>grades I-IV.
<sup>b</sup>extensive chronic GVHD.
<sup>c</sup>before donor lymphocyte infusions given in 36 of 88 (41%) patients,
<sup>d</sup>in patients with low-grade NHL,
<sup>e</sup>in patients with high-grade NHL,
<sup>f</sup>in patients with good-risk diseases,
<sup>g</sup>in patients with high-risk diseases,
<sup>h</sup>the clinical trials were carried out jointly by a group of collaborators located at the Fred Hutchinson Cancer Research Center, University of Washington, Children’s Hospital and Regional Medical Center, and Veterans Administration Medical Center, all in Seattle, Washington, U.S.A.; Stanford University, Palo Alto, California, U.S.A.; City of Hope National Medical Center, Duarte, California, U.S.A.; University of Leipzig, Germany; University of Colorado, Denver, Colorado, U.S.A.; University of Torino, Italy; University of Arizona, Tucson, Arizona, U.S.A.; Baylor University, Dallas, Texas, U.S.A.; University of Utah, Salt Lake City, Utah, U.S.A.; Oregon Health Sciences University, Portland, Oregon, U.S.A.; the Medical College of Wisconsin, Milwaukee, Wisconsin, U.S.A.; and Emory University, Atlanta, Georgia, U.S.A.

**Abbreviations:** NRM, non-relapse mortality; ATG, antithymocyte globulin; CSP, cyclosporine; FK506, tacrolimus; MTX, methotrexate; pts, patients; OS, overall survival; DFS, disease-free survival; PFS, progression-free survival; NR, not reported.

**Source:** From Ref. 19.
NONMYELOABLATIVE OR REDUCED-INTENSITY REGIMENS

Many of the reduced-intensity conditioning regimens do not meet criteria of nonmyeloablative conditioning as first proposed by Champlin et al., which include (i) no eradication of host hematopoiesis, (ii) prompt hematologic recovery (<4 weeks) without transplant, and (iii) presence of mixed chimerism upon engraftment (18,19). Most reduced-intensity conditioning regimens combine modest dose of highly immunosuppressive purine analogs (fludarabine, cladribine, or pentostatin) given to overcome host-versus-graft reactions, with reasonably high-dose of alkylating agents, usually busulfan or melphalan, given to supplement the GVT effects in the task of tumor eradication. Conversely, nonmyeloablative conditioning regimens usually combine two highly immunosuppressive agents together (low-dose TBI, fludarabine, or cyclophosphamide) to overcome host-versus-graft reactions to allow engraftment and tumor eradication via GVT effects (16,20). Although the division of what constitutes a nonmyeloablative versus reduced-intensity conditioning regimen is somewhat arbitrary, the distinction might be important, given that nonmyeloablative conditioning has been associated with a lower degree of donor engraftment, decreased risk of nonrelapse mortality, and perhaps higher risk of relapse in comparison with reduced-intensity regimens (21).

NONMYELOABLATIVE CONDITIONING WITH 2 GY TBI AND FLUDARABINE

On the basis of preclinical studies in a canine model (22), we developed a nonmyeloablative conditioning regimen for allogeneic HCT consisting of 2 Gy TBI given on day 0, with postgrafting immunosuppression combining mycophenolate mofetil (MMF) and cyclosporin (CSP) (16). Nine of the first 44 patients (20%, including four of eight patients with chronic myeloid leukemia) given this regimen had nonfatal graft rejections (16,23). In order to reduce the risk of graft rejection, fludarabine 30 mg/m²/day × 3 days was added to the 2 Gy TBI, and the rejection rate decreased to 3% (24). The same nonmyeloablative regimen combining fludarabine and 2 Gy TBI was used to condition patients with 10/10-human leukocyte antigen (HLA)-matched unrelated donors (25). Sustained engraftment was observed in 60 of 71 (85%) peripheral blood stem cells (PBSC) recipients and in 10 of 18 (56%) marrow recipients. On the basis of this observation, all subsequent unrelated recipients were given PBSC grafts. Analysis of the first 451 patients with hematologic malignancies transplanted in a multicenter international consortium is shown in Table 1 (24). Median patient age was 55 (range, 5–74) years, and median follow-up was 696 (range, 82–1795) days. All patients were deemed ineligible for myeloablative conditioning because of age and/or comorbidities. Diagnoses included multiple myeloma (n = 114), myelodysplastic syndromes or myeloproliferative disorders (n = 82), NHLs (n = 79), acute myeloid leukemia (n = 59), chronic lymphocytic leukemia
(n = 44), chronic myeloid leukemia (n = 37), Hodgkin’s disease (n = 26), and acute lymphoblastic leukemia (n = 10). Three hundred and thirty-two patients had measurable disease at transplantation, and 56.5% achieved complete (49%) or partial (7.5%) remissions. The incidences of nonrelapse mortality at 100 days and two years were 7% and 22%, respectively. Main causes of nonrelapse mortality were GVHD and infections. The two-year probabilities of overall and progression-free survivals were 51% and 37%, respectively.

**KINETICS OF DONOR ENGRAFTMENT AFTER NONMYELOABLATIVE CONDITIONING**

The engraftment kinetics after nonmyeloablative conditioning regimen were first analyzed by Childs et al. (17). The authors studied chimerism (i.e., proportion of hematopoietic cells of donor origin) evolution in 15 patients conditioned with fludarabine (125 mg/m²) and cyclophosphamide (120 mg/kg). The patterns of engraftment varied between patients, but most often, full donor chimerism was achieved earlier among T-cells than among granulocytes, and achievement of full donor T-cell chimerism preceded GVHD and antitumor responses. Conversely, Ueno et al. studied chimerism evolution in 23 patients with metastatic tumors transplanted after conditioning with fludarabine (125–150 mg/m²) and melphalan (140 mg/m²) (26). All patients had full donor T-cell and granulocyte chimerisms by day 30 after HCT.

We analyzed the kinetics of donor engraftment in various peripheral blood hematopoietic subpopulations from 120 patients conditioned with 2 Gy TBI +/- fludarabine and postgrafting immunosuppression with MMF and CSP (27). On day 14 post transplant, the highest degree of donor chimerism was seen in the NK cells followed by T-cells, monocytes, and granulocytes (Fig. 2A). By day 28, donor granulocyte chimerism had surpassed those in the remaining cell populations. PBSC recipients had higher degrees of donor T-cell chimerism than recipients of marrow, while greater intensity of therapy before HCT was associated with higher degrees of donor chimerisms. Day-14 donor chimerism levels less than 50% among T-cells (p = 0.0007) and NK cells (p = 0.003) predicted graft rejection (Fig. 2B). High donor chimerism levels on day 14 among T-cells were associated with increased risks of grades II to IV acute GVHD (p = 0.02), while high donor T-cell (p = 0.002) and NK cell (p = 0.002) chimerism levels from days 14 to 42 were associated with decreased risks of relapse. In addition, high levels of donor NK cell chimerism early after HCT correlated with better progression-free survival (p = 0.02) and a trend for better overall survival (p = 0.09).

These observations suggest that assessing donor chimerism levels among T-cells and NK cells might help identify patients at risk for graft rejection, acute GVHD, and relapse, and thereby allow early interventions with DLI and/or immunosuppressive drugs (7,28).
GVHD AND GVT EFFECTS AFTER NONMYELOABLATIVE CONDITIONING

GVHD remains a major cause of morbidity and mortality after nonmyeloablative or reduced-intensity conditioning (16,25). Mielcarek et al. compared GVHD in 52 patients given myeloablative conditioning with that among 44 patients given nonmyeloablative conditioning (29). Recipients in both groups were age matched, with median ages of 54 years in the myeloablative and 56 years in the nonmyeloablative groups. Grafts were from either related or unrelated donors who were serologically matched for HLA-A, -B, and -C and allele level matched for HLA-DRB1 and -DQB1. Postgrafting immunosuppression consisted of methotrexate (MTX) plus CSP (n = 45) or MMF plus CSP (n = 7) in myeloablative
recipients, versus MMF plus CSP in all nonmyeloablative recipients. The cumulative incidences of grades II to IV acute GVHD were 85% in myeloablative recipients versus 64% in nonmyeloablative recipients \( (p = 0.001) \), but there were no differences in the cumulative incidences of extensive chronic GVHD \( (71\% \text{ vs. } 73\%) \), respectively. The 15-month cumulative incidences of death with manifestations of GVHD under treatment were 35% and 24% in myeloablative and nonmyeloablative recipients, respectively \( (\text{NS}) \).

Although there is a close relationship between GVHD and GVT responses observed after myeloablative HCT \( (2–4, 6) \), whether some degree of clinical GVHD was required for accomplishing remissions after nonmyeloablative conditioning was less clear. In order to address this question, we analyzed the impact of either acute or chronic GVHD on HCT outcomes in 322 patients with hematologic malignancies given grafts from HLA-matched related \( (n = 192) \) or unrelated \( (n = 130) \) donors following conditioning with 2 Gy TBI with or without fludarabine \( (90 \text{ mg/m}^2) \) \( (20) \). Two hundred and twenty-one patients had measurable malignant disease at the time of transplantation, and 126 of them \( (57\%) \) achieved complete \( (n = 98) \) or partial \( (n = 28) \) remissions 27 to 963 days \( \text{median, 176 days} \) after HCT. Extensive chronic GVHD was suggestively associated with a higher probability of achieving complete remissions \( \text{HR 1.7, } p = 0.07 \) \), but no associations between acute GVHD and achievement of complete remissions were seen. Grades II and III to IV acute GVHD did not decrease the risks of progression/relapse but were associated with an increased risk of nonrelapse mortality and decreased progression-free survival. In contrast, extensive chronic GVHD was associated with decreased risk of progression/relapse \( \text{HR 0.4, } p = 0.006 \) \) and better progression-free survival \( \text{HR 0.5, } p = 0.003 \) \) \( \text{Fig. 3} \). The beneficial impact of chronic GVHD on relapse was seen in all disease groups but was strongest in the group of patients with acute myeloid leukemia or myelodysplastic syndrome \( \text{HR 0.2, } p = 0.0009 \)\

Similarly, a number of other recent reports have shown a negative impact of grades II to IV acute GVHD \( (30, 31) \) but a beneficial impact of chronic GVHD \( (31, 32) \) on HCT outcomes in patients given HCT after reduced-intensity or nonmyeloablative conditioning.

Some reduced-intensity conditioning regimens have used in vivo T-cell depletion of the grafts \( \text{with either antithymocyte globulin (ATG) or alemtuzumab} \) to decrease the incidence of GVHD. While these strategies achieved their goal \( (11, 13) \), increased incidences of both infections and disease relapses were observed, resulting in comparable progression-free survival.

TOXICITIES AFTER MYELOABLATIVE OR NONMYELOABLATIVE CONDITIONING

A number of retrospective studies have compared incidences of toxicity and infection after nonmyeloablative versus myeloablative conditioning \( \text{Table 3} \) \( (33–37) \). Nonmyeloablative conditioning was associated with decreased
transfusion requirements (33), decreased incidence of idiopathic pneumonia syndrome (IPS) (34), decreased incidence of sinusoidal obstruction syndrome (SOS) (35), decreased incidence of acute renal failure (38), and decreased incidence of bacterial and cytomegalovirus (CMV) infections early after HCT (36,37). However, overall CMV reactivations and fungal infections were similarly frequent after nonmyeloablative and myeloablative conditioning (36,37).

Sorror et al. analyzed transplantation-related toxicities (graded according to the National Cancer Institute common toxicity criteria) following HLA-matched unrelated HCT in 134 concurrent patients given either nonmyeloablative ($n = 60$) or myeloablative ($n = 74$) conditioning (39). Additionally, the effects of pre-transplant comorbidities [graded according to the Charlson Comorbidity Index (CCI) score] on outcome were investigated. Lower numbers of gastrointestinal ($p < 0.0001$), hepatic ($p = 0.005$), hematologic ($p < 0.0001$), infection-related ($p = 0.02$), and hemorrhagic ($p = 0.02$) grades III to IV toxicities were seen in nonmyeloablative compared with myeloablative recipients, whereas incidences of cardiovascular, metabolic, pulmonary, and renal toxicities were not statistically significantly different between the two groups. The one-year nonrelapse mortality was 32% in patients given myeloablative conditioning compared with 20% in patients given nonmyeloablative conditioning. In multivariate analyses adjusting for disease risk, age, and CCI score at HCT, myeloablative conditioning was
associated with increased risks of grade IV toxicities (HR 9.4, $p = 0.0001$) and higher one-year nonrelapse mortality (HR 3.0, $p = 0.04$). Interestingly, higher pretransplant CCI scores predicted for increased mortality. Comparable results were observed by Diaconescu et al. in patients given grafts from HLA-identical sibling donors (40).

### Table 3: Toxicity After Nonmyeloablative (Consisting of 2 Gy Total Body Irradiation with or Without 90 mg/m² Fludarabine) Vs. Myeloablative Conditioning Regimens

<table>
<thead>
<tr>
<th>Toxicity (Refs.)</th>
<th>Nonmyeloablative</th>
<th>Myeloablative</th>
<th>$p$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematological Toxicity (transfusion requirements) (33)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median units red cells</td>
<td>2 (0–50)</td>
<td>6 (0–34)</td>
<td>$p = 0.0002$</td>
</tr>
<tr>
<td>Median units platelets</td>
<td>0 (0–214)</td>
<td>24 (4–358)</td>
<td>$p &lt; 0.0001$</td>
</tr>
<tr>
<td>Pulmonary Toxicity (34)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>120-day CI of IPS</td>
<td>2.2%</td>
<td>8.4%</td>
<td>$p = 0.003$</td>
</tr>
<tr>
<td>Hepatic Toxicity (35,40)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200-day CI of hyperbilirubinemia$^a$</td>
<td>26%</td>
<td>48%</td>
<td>ND</td>
</tr>
<tr>
<td>100-day CI of sinusoidal obstructive syndrome</td>
<td>0%</td>
<td>18%</td>
<td>ND</td>
</tr>
<tr>
<td>Renal toxicity (38)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100-day CI of grades II–III acute renal failure</td>
<td>47%</td>
<td>73%</td>
<td>$p &lt; 0.0001$</td>
</tr>
<tr>
<td>100-day CI of dialysis</td>
<td>3%</td>
<td>12%</td>
<td>$p &lt; 0.0001$</td>
</tr>
<tr>
<td>Infections (36,37)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>30-day CI of bacterial infection</td>
<td>9%</td>
<td>27%</td>
<td>$p = 0.01$</td>
</tr>
<tr>
<td>100-day CI of bacterial infection</td>
<td>27%</td>
<td>41%</td>
<td>$p = 0.07$</td>
</tr>
<tr>
<td>1-yr CI of aspergillosis</td>
<td>14%</td>
<td>10%</td>
<td>$p = 0.30$</td>
</tr>
<tr>
<td>100-day CI of CMV disease</td>
<td>6%</td>
<td>19%</td>
<td>$p = 0.06$</td>
</tr>
<tr>
<td>1-yr CI of CMV disease</td>
<td>24%</td>
<td>25%</td>
<td>$p = 0.87$</td>
</tr>
<tr>
<td>GVHD (29)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100-day CI of grades II–IV acute GVHD</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Matched siblings</td>
<td>62%</td>
<td>77%</td>
<td>$p = 0.02$</td>
</tr>
<tr>
<td>Matched unrelated donors</td>
<td>65%</td>
<td>95%</td>
<td>$p = 0.01$</td>
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<tr>
<td>CI of extensive chronic GVHD</td>
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<td></td>
<td></td>
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<tr>
<td>Matched siblings</td>
<td>77%</td>
<td>74%</td>
<td>$p = 0.37$</td>
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<tr>
<td>Matched unrelated donors</td>
<td>68%</td>
<td>69%</td>
<td>$p = 0.37$</td>
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<tr>
<td>Mortality from GVHD</td>
<td>24%</td>
<td>35%</td>
<td>$p = 0.07$</td>
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</table>

$^a$4 mg/dL.

**Abbreviations:** CI, cumulative incidence; IPS, idiopathic pneumonia syndrome; ARF, acute renal failure; CMV, cytomegalovirus; GVHD, graft-versus-host disease.
RELAPSE AND SURVIVAL AFTER MYELOABLATIVE OR NONMYELOABLATIVE CONDITIONING

It has remained difficult to compare relapse risk and survival after myeloablative versus nonmyeloablative recipients, given the short follow-up (and relatively low number) of patients given HCT after nonmyeloablative conditioning so far and the fact that nonmyeloablative recipients were generally older and had more comorbidities than patients given myeloablative conditioning. Two randomized studies in the early 1990s demonstrated lower risk of relapse, increased non-relapse mortality, and similar survival in patients treated with cyclophosphamide and 15.5 Gy versus 12 Gy TBI followed by HLA-identical sibling HCT, demonstrating that dose intensity does matter for both toxicity and antitumor efficacy (41,42).

Alyea et al. performed a retrospective analysis of 152 patients (>50 years old) with hematologic malignancies undergoing HCT after myeloablative [mainly cyclophosphamide (3.6 g/m²) and TBI (14 Gy)] or reduced-intensity conditioning combining fludarabine (120 mg/m²) and intravenous busulfan (3.2 mg/kg) (43). Patients given nonmyeloablative conditioning were more likely to receive grafts from unrelated donors (58% vs. 36%, \( p = 0.009 \)), to have received a prior HCT (25% vs. 4%, \( p < 0.0001 \)), and to have active disease at the time of transplantation (85% vs. 59%, \( p < 0.001 \)). With a median follow-up of 18 months, the cumulative incidences of relapse and nonrelapse mortality were 46% and 32% in the reduced-intensity conditioning group, versus 30% and 50%, respectively, in the myeloablative group. Two-year overall survival was perhaps superior in the nonmyeloablative group (39% vs. 29%; \( p = 0.056 \)).

RESULTS IN SPECIFIC DISEASES

Acute Myeloid Leukemia and Myelodysplastic Syndrome

Hegenbart et al. analyzed outcome of 122 patients with acute myeloid leukemia ineligible for conventional HCT given allogeneic grafts after 2 Gy TBI with or without added fludarabine (90 mg/m²), and postgrafting immunosuppression combining MMF and CSP (44). Two-year probabilities of overall survival were 51% for patients transplanted in first complete remission (\( n = 51 \)), 61% for those transplanted in second remission (\( n = 39 \)), and 28% for those transplanted beyond second remission (\( n = 32 \)) (Fig. 4). High cytogenetic risks predicted for decreased overall survival (HR 2.4, \( p = 0.008 \)).

Using a genetic randomization through a “donor” versus “no donor” comparison, Mohty et al. investigated whether allogeneic HCT after conditioning with fludarabine (180 mg/m²), busulfan (8 mg/kg), and ATG increased survival in adults (median age 52 years) with newly diagnosed high-risk acute myeloid leukemia in first complete remission ineligible for conventional HCT (45). Ninety-five patients were retrospectively analyzed; 35 had an HLA-identical sibling donor (donor group), while 60 had no related HLA-matched donor (no donor group).
Twenty-five of thirty-five patients included in the donor group (71%) received allogeneic HCT. The 10 remaining patients with an identified donor did not receive allogeneic HCT because of patient or donor refusal (n = 6), early relapse (n = 2), or psychiatric disorders appearing before HCT (n = 2). In an intention to

Figure 4  Example of GVT response in a patient with mantle cell lymphoma relapsing after high-dose radiolabeled antibodies with autologous peripheral blood stem cell support. (A) Pretreatment CT scan image (day 27) through the upper pelvis demonstrating an 8-cm by 7-cm mass that extended through 12 0.5-cm cuts. (B) CT scan image through the same region demonstrating complete resolution of the mass on day +74 after nonmyeloablative transplantation from a matched unrelated donor. The patient remains in remission 30 months after transplantation with no evidence of GVHD. Abbreviations: GVT, graft-versus-tumor; CT, computed tomography; GVHD, graft-versus-host disease. Source: From Ref. 50.
treat analysis, the four-year probability of progression-free survivals was 54% in
the donor group versus 30% in the nondonor group \( p = 0.01 \).

Ho et al. analyzed data from 62 patients with myelodysplastic syndrome
given allografts from related \( n = 24 \) or unrelated \( n = 38 \) donors after reduced-
intensity conditioning with fludarabine \( 150 \text{ mg/m}^2 \), oral busulfan \( 8 \text{ mg/kg} \),
and alemtuzumab \( 100 \text{ mg total dose} \) \( 46 \). Postgrafting immunosuppression
consisted of CSP alone. Median patient age at HCT was 56 years for patients
given grafts from siblings and 52 years for patients given grafts from unrelated
donors. Sixteen patients had refractory anemia, 19 refractory anemia with blast
excess, 23 refractory anemia with blast excess in transformation, and 4 chronic
myelomonocytic leukemia. The one-year probabilities of nonrelapse mortality,
overall survival, and progression-free survival were 5%, 73%, and 61%,
respectively, for patients given grafts from related donors versus 21%, 71%, and
59%, respectively, for patients given grafts from unrelated donors.

**Chronic Myeloid Leukemia**

Or et al. reported data from 24 patients (median age 35 years) with chronic
myeloid leukemia in first chronic phase given HLA-matched related \( n = 19 \) or
unrelated \( n = 5 \) grafts after reduced-intensity conditioning combining fludar-
abine \( 180 \text{ mg/m}^2 \), busulfan \( 8 \text{ mg/kg} \), and ATG \( 11 \). Day-100 mortality was
0\%, but three patients died as a consequence of GVHD 116, 499, and 726 days
after HCT. The five-year probability of progression-free survival was 85%, with
all 21 survivors having negative reverse transcriptase-polymerase chain reaction
(RT-PCR) for Bcr-Abl.

Kerbauy et al. analyzed data from 24 patients (median age 58 years) with
chronic myeloid leukemia in first chronic phase \( n = 14 \) or beyond \( n = 10 \)
given PBSC from HLA-matched related donors after conditioning with 2 Gy TBI
with \( n = 16 \) or without \( n = 8 \) fludarabine \( 23 \). Four of eight patients not
given fludarabine experienced nonfatal graft rejection and recurrence of chronic
myeloid leukemia, while the 20 remaining patients achieved sustained engraft-
ment. The two-year overall survival rate was 70% for patients transplanted in
first chronic phase, and 56% for those with more advanced disease. Nine of ten
patients transplanted in first chronic phase after conditioning with 2 Gy TBI with
fludarabine achieved molecular remissions 3 to 24 months after HCT.

In contrast to what was observed in patients given grafts from HLA-
matched sibling donors, a high rate of graft rejection among chronic myeloid
leukemia patients receiving grafts from unrelated donors after nonmyeloablative
or reduced-intensity conditioning has been reported. We observed graft rejection
in 9 of 21 patients given unrelated grafts for chronic myeloid leukemia after 2 Gy
TBI and fludarabine \( 47 \). Graft rejections were nonfatal in all cases and fol-
lowed by autologous reconstitution with persistence or recurrence of chronic
myeloid leukemia. Seven of eleven patients with sustained engraftment,
including all five patients in first chronic phase were alive in complete
cytogenetic remissions 118–1205 (median 867) days after HCT. Hallemeier et al. observed graft failure in 5 of 22 evaluable patients given unrelated grafts after conditioning with 5.5 Gy TBI and cyclophosphamide (120 mg/kg) (14). Further efforts for reducing the risk of graft rejection in patients with chronic myeloid leukemia given unrelated HCT are directed at increasing the degree of pre-transplant immunosuppression.

Lymphoma and Chronic Lymphocytic Leukemia

Khouri et al. reported results in 20 patients (median age 51 years) with low-grade NHL given grafts from siblings after conditioning with fludarabine (90–125 mg/m²) and cyclophosphamide (2000–2250 mg/m²), with or without added rituximab (15). Postgrafting immunosuppression consisted of tacrolimus and MTX. After a median follow-up of 21 months, the two-year current probability of disease-free survival was 84%. The same authors evaluated the efficacy of nonmyeloablative HCT in 20 patients with NHL recurrence after autologous HCT (48). Ten patients achieved complete remission with salvage chemotherapy before non-ablative HCT, eight had a partial response, and two had stable disease. One patient died at 10.5 months from a fungal infection. The three-year progression-free survival was 95%.

Robinson et al. analyzed data from 188 patients (median age 40 years) with lymphoma (low-grade NHL (n = 52), high-grade NHL (n = 62), mantle cell lymphoma (n = 22), or Hodgkin’s disease (n = 52)) given HCT after various reduced-intensity or nonmyeloablative conditioning in EBMT-affiliated centers (49). The one-year probabilities of nonrelapse mortality were 39% and 22% in patients older or younger than 50 years, respectively (p = 0.03). The two-year probabilities of overall and progression-free survival were 65% and 54% for patients with low-grade NHL, 47% and 13% for patients with high-grade NHL, 13% and 0% for patients with mantle cell lymphoma, and 56% and 42%, respectively, for patients with Hodgkin’s disease. Chemosensitive disease at HCT was associated with better overall (RR, 2.4; p = 0.002) and progression-free (RR, 2.3; p = 0.007) survivals in multivariate analyses.

Morris et al. reported results of 88 patients with NHL given allogeneic HCT after conditioning with fludarabine (150 mg/m²), melphalan (140 mg/m²), and alemtuzumab (100 mg) (13). Sixty-five patients received PBSC from HLA-identical siblings, while 23 received bone marrow from matched unrelated donors. GVHD prophylaxis consisted of CSP alone. Before DLI, grades III to IV acute GVHD were seen in four patients, but two additional patients developed grade IV acute GVHD after DLI. The actuarial three-year probability of current progression-free survival was 65% for patients with low-grade lymphoma (n = 41), 50% for patients with mantle cell lymphoma (n = 10), and 34% for patients with high-grade lymphoma (n = 37) (Table 2).

Maris et al. analyzed outcomes of 33 patients with relapsed or refractory mantle cell lymphoma who underwent allogeneic HCT from related (n = 16) or
unrelated \((n = 17)\) donors after 2 Gy TBI and fludarabine \((90 \text{ mg/m}^2)\) (50). The overall response rate in the 20 patients with measurable disease at the time of HCT was 85\% (including 75\% complete remissions and 10\% partial remissions) (Fig. 4). The two-year probabilities of relapse, nonrelapse mortality, and progression-free survival were 9\%, 24\%, and 60\%, respectively.

Sorror et al. described outcomes in 64 patients with chronic lymphocytic leukemia (median age 56 years) given HCT from HLA-matched related \((n = 44)\) or unrelated \((n = 20)\) donors after conditioning consisting of 2 Gy TBI with \((n = 53)\) or without \((n = 11)\) fludarabine \((90 \text{ mg/m}^2)\) (51). Eighty-eight percent of patients were refractory to fludarabine. With a median follow-up of 24 months, the overall response rate was 67\% (including 50\% with complete remission). The two-year rates of nonrelapse mortality, overall, and progression-free survivals were 22\%, 60\%, and 52\%, respectively. Bulky lymphadenopathy (lymph node diameter $\geq 5 \text{ cm}$) independently predicted higher incidence of relapse/progression \((HR 3.8, p = 0.009)\), while marrow infiltration with more than 50\% leukemic cells was associated with worse survival \((HR 2.4, p = 0.05)\). These data, in agreement with those described in smaller series (52,53), show that chronic lymphocytic leukemia is remarkably susceptible to GVT effects.

**Multiple Myeloma**

Crawley et al. reported data from 229 patients given allogeneic HCT after various reduced-intensity conditioning in EBMT-affiliated centers (32). One hundred and ninety-two patients received grafts from related donors and 37 from unrelated donors. Overall, 25\% and 48\% of patients achieved complete or partial remissions, respectively. The three-year probabilities of overall and progression-free survivals were 41\% and 21\%, respectively. Adverse progression-free survival was associated with alemtuzumab-containing conditioning \((RR 1.8, p = 0.001)\) and chemoresistance prior to transplant \((RR 2.4, p = 0.0004)\), suggesting that heavily pretreated patients and those with progressive disease did not benefit from this approach. Chronic GVHD was associated with better progression-free survival \((p < 0.0001)\), while grades III to IV acute GVHD was associated with a worse overall survival \((p = 0.0007)\) and did not decrease the risk of relapse.

**TANDEM AUTOLOGOUS/ALLOGENEIC HCT**

To allow older patients with aggressive chemosensitive disease to benefit from both high-dose chemotherapy and GVT effects, it has been proposed to first use high-dose conditioning and autologous transplantation, which can be administered with overall mortality rates of less than 5\%, followed one to three months later by allogeneic HCT using nonmyeloablative conditioning (tandem autologous/allogeneic HCT). This strategy, pioneered by Carella et al. in patients with refractory lymphoma (54), was evaluated by Maloney et al. in 54 patients with
multiple myeloma. Patients were first given autologous HCT after a cyto-
eductive dose of 200-mg/m² melphalan; this was followed 40 to 229 (median 62)
days later by allogeneic HCT after 2 Gy TBI (55). Patients were 29 to 71
(median 52) years old, and 48% had refractory (35%) or relapsed (13%) disease.
Remarkably, the 100-day mortalities after autologous and allogeneic HCT were
2% each, contrasting with the high nonrelapse mortality (ranging from 20% to
50% (56) observed in patients with multiple myeloma given allogeneic HCT
after myeloablative conditioning. The two-year overall and progression-free
survivals were 78% and 55%, respectively.

CONCLUSIONS AND CLINICAL PERSPECTIVES
FOR THE NEXT FIVE YEARS
Reduced-intensity conditioning and nonmyeloablative regimens have allowed
engraftment of allogeneic hematopoietic cells and the development of GVT
effects. Antitumor responses have generally required extended periods of time,
with a median time of six months required before achievement of complete
remissions. In patients with slowly progressing diseases such as chronic myeloid
leukemia in first chronic phase, low-grade myelodysplastic syndrome, chronic
lymphocytic leukemia, or low-grade NHL, or with more aggressive diseases in
complete remission, nonmyeloablative conditioning may be sufficient to achieve
cure of the disease. A number of approaches are being explored for patients with
more aggressive diseases, such as acute leukemias, high-grade myelodysplastic
syndrome, multiple myeloma, or high-grade lymphomas, who are not in com-
plete remission.

A first approach is to combine nonmyeloablative HCT with “disease-
targeted” therapy, such as monoclonal antibodies or thalidomide. Khouri et al.
reported 17 patients with chronic lymphocytic leukemia given allogeneic grafts
from related donors after fludarabine (90 mg/m²) and cyclophosphamide
(2250 mg/m²) (53). Ten patients received rituximab in addition to chemotherapy.
The two-year overall survivals were 100% and 57%, in patients given or not
given rituximab, respectively. We have been studying the administration of I-131
anti-CD45 monoclonal antibody followed by 2 Gy TBI and fludarabine to
condition patients with acute myeloid leukemia not in remission and patients
with advanced myelodysplastic syndrome (57). This approach has allowed
administration of 40 Gy to marrow and 56 Gy to spleen, with a relative sparing
of nonhematopoietic organs. Kroger et al. investigated the efficacy of thalido-
mide (100 mg) combined with DLI in 18 patients with multiple myeloma pro-
gressing after reduced-intensity HCT (58). The overall response rate was 67%,
including 22% complete remissions. No grades II to IV acute GVHD were seen,
while de novo limited chronic GVHD occurred in two patients (11%). The two-
year progression-free survival after DLI was 84%.

A second approach might consist of posttransplant infusion of donor-
specific cytotoxic T-cells directed against either tumor antigens (such as
proteinase 3 or Wilms’ tumor-suppressor 1 in case of leukemia or patient paraprotein in case of multiple myeloma) or recipient minor histocompatibility antigens expressed exclusively on hematopoietic cells (such as HA-1 and HA-2 minor histocompatibility antigens), potentially increasing antitumoral efficacy of DLI with a low risk of inducing GVHD (59).

A number of prospective phase III studies aimed at better defining the role of nonmyeloablative conditioning in patients with multiple myeloma (BMT-CTN 01–02), lymphoma (BMT-CTN 02–02), or acute myeloid leukemia (GOELAMS AML 2001, FHCRC-1992.00) are ongoing in the United States and in Europe. Other randomized studies are focusing on comparing different conditioning regimens (FHCRC-1813.00) or defining the best postgrafting immunosuppression in the nonmyeloablative transplantation setting (FHCRC-1938.00).

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REFERENCES


**BOOK: DK0832_Kaspers**

**CHAPTER 24**

**TO: CORRESPONDING AUTHOR**

**AUTHOR QUERIES - TO BE ANSWERED BY THE AUTHOR**

The following queries have arisen during the typesetting of your manuscript. Please answer these queries.

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