Kinetics of engraftment following allogeneic hematopoietic cell transplantation with reduced-intensity or nonmyeloablative conditioning

Frédéric Baron, Marie-Térèse Little, Rainer Storb

Summary Nonmyeloablative or reduced-intensity conditioning regimens have been used to condition elderly or ill patients with hematological malignancies for allogeneic hematopoietic cell transplantation (HCT). Initial mixed donor/host chimerism (i.e. the coexistence of hematopoietic cells of host and donor origin) has been observed in most patients after such transplants. Here, we describe both factors affecting engraftment kinetics in patients given a nonmyeloablative or a reduced-intensity conditioning, and associations between peripheral blood cell subset chimerism levels and HCT outcomes.

Reduced-intensity and nonmyeloablative conditioning regimens for allogeneic HCT

To avoid serious regimen-related toxicities, the use of conventional allogeneic hematopoietic cell transplantation (HCT) has been restricted to younger and medically fit patients. This is unfortunate since the median age at diagnosis for patients with hematological malignancies such as acute and chronic leukemias, lymphomas, multiple myeloma or myelodysplastic syndromes, ranges from 65 to 70 years, thereby precluding the use of allogeneic HCT for most patients with these diseases. The curative potential of allogeneic HCT has been ascribed not only to the eradication of malignant cells by high-dose chemotherapy and total body irradiation, but also to immune-mediated graft-versus-tumor (GVT) effects. The power of the
<table>
<thead>
<tr>
<th>Center</th>
<th>Preparative regimens</th>
<th>Postgraft immuno-suppression</th>
<th>No. of patients (median age in years)</th>
<th>Diseases</th>
<th>GVHD</th>
<th>NRM (days after transplant)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>MD Anderson</td>
<td>Fludarabine 25 mg/m²/day (or 2-CDA 12 mg/m²)×5 days Melphalan 140–180 mg/m²</td>
<td>FK506 + MTX</td>
<td>86 (52)</td>
<td>Hematological malignancies</td>
<td>49%</td>
<td>68%</td>
<td>37% (at 100 days)</td>
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<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>730-day DFS: 23%</td>
</tr>
<tr>
<td>United Kingdom</td>
<td>Fludarabine 30 mg/m²/day×5 days</td>
<td>CSP +/− MTX</td>
<td>44 (41)</td>
<td>Hematological malignancies, 19 pts had a previous failed transplant</td>
<td>3/44.1 after DLI, NR 11% (at 365 days)</td>
<td>11% (at 365 days)</td>
<td>360-day OS: 73%</td>
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<td></td>
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<td></td>
<td>360-day PFS: 71%</td>
</tr>
<tr>
<td>Jerusalem</td>
<td>Fludarabine 30 mg/m²/day×6 days Busulfan (p.o.) 4 mg/kg/day×2 days ATG 5–10 mg/kg/day×4 days Melphalan 140 mg/m² CAMPATH-1H 20 mg/day×5 days</td>
<td>CSP +/− MTX</td>
<td>24 (35)</td>
<td>Chronic myeloid leukemia in first chronic phase</td>
<td>75%a</td>
<td>55%</td>
<td>3 pts (days 116, 499 and 726)</td>
</tr>
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<td></td>
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<td></td>
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<td></td>
<td></td>
<td></td>
<td>11 pts were surviving free of progression</td>
</tr>
<tr>
<td>National Institutes of Health</td>
<td>Fludarabine 25 mg/m²/day×5 days Cyclophosphamide 1 g/m²/day×2 days or 750 mg/m²/day×3 days ±Rituximab</td>
<td>CSP</td>
<td>15 (50)</td>
<td>Hematological +solid malignancies</td>
<td>10/15 pts. 1 after DLI NR 2 pts (days 59 and 205)</td>
<td>2 pts (days 77 and 180)</td>
<td>8/15 pts survived between 121 and 409 (median, 200) days</td>
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<td></td>
<td></td>
<td>11 pts were surviving</td>
</tr>
<tr>
<td>Boston</td>
<td>Cyclophosphamide 50 mg/kg/day×3–4 days ATG 30 mg/kg/day×3 days or 15 mg/kg/day×4 days Thymic irradiation 7 Gy²</td>
<td>CSP</td>
<td>21 (44)</td>
<td>Hematological malignancies</td>
<td>12 pts. 6 after DLI NR</td>
<td>2 pts (days 77 and 180)</td>
<td>At a median follow-up of 445 days: 11 pts were surviving 7 pts were surviving free of progression</td>
</tr>
</tbody>
</table>
lymphocyte-mediated GVT effects has led several groups of investigators to explore the curative potential of donor lymphocyte infusions (DLI) in patients who had relapsed with hematological malignancies after allogeneic HCT. The induction of durable remissions by DLI in a number of patients demonstrated that GVT effects were capable of eradicating hematological malignancies, even in the absence of chemotherapy.

In an attempt to extend the use of allogeneic HCT to older patients and those with comorbid conditions, several groups of investigators have explored allogeneic HCT after reduced-intensity or truly nonmyeloablative conditioning regimens in which the burden of tumor eradication was shifted toward GVT effects. Examples of reduced-intensity or truly nonmyeloablative conditioning regimens are shown in Table 1. Many of the regimens did not meet criteria of nonmyeloablative conditioning which have included: (1) no eradication of host hematopoiesis, (2) prompt endogenous hematologic recovery (<4 weeks) without transplant and (3) presence of mixed chimerism upon allogeneic engraftment. Analogous to conventional regimens, reduced-intensity regimens produce major anti-tumor effects and reduce host-versus-graft reactions. In contrast, nonmyeloablative regimens rely on optimization of pre- and post-transplant immunosuppression to overcome host-versus-graft reactions and allow allogeneic engraftment, thereby setting the stage for eradication of tumors by GVT effects. In patients with slowly progressing diseases (e.g. chronic lymphocytic leukemia, low-grade nonHodgkin lymphoma, or chronic myeloid leukemia in first chronic phase) or with more aggressive diseases in complete remission, a nonmyeloablative conditioning regimen might be sufficient to achieve engraftment and cure the malignant disease. However, cytoreduction might be required in patients with aggressive diseases, e.g. acute leukemia, multiple myeloma, high-grade lymphoma, Hodgkin disease, who are not in complete remission at the time of the transplant.

After extensive pre-clinical studies in a dog model, we have developed a nonmyeloablative conditioning regimen consisting of low-dose (2 Gy) total body irradiation (TBI) ± fludarabine 30 mg/m²/day × 3 days to condition elderly or ill patients with hematological malignancies for allogeneic HCT. Postgrafting immunosuppression consisted of mycophenolate mofetil (MMF) and cyclosporine (CSP) (Fig. 2). 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, WA, USA; Stanford University, Palo Alto, CA, USA; City of Hope National Medical Center, Duarte, CA, USA; University of Leipzig, Germany; University of Colorado, Denver, CO, USA; University of Torino, Italy; University of Arizona, Tucson, AZ, USA; Baylor University, Dallas, TX, USA; University of Utah, Salt Lake City, UT, USA; Oregon Health Sciences University, Portland, OR, USA; and, more recently, the Medical College of Wisconsin, Milwaukee, WI, USA; and Emory University, Atlanta, GA, USA. The transplant regimen was remarkably well tolerated, with the majority of patients receiving their transplants in the outpatient setting.\textsuperscript{12,16,17}

**Mixed chimerism after allogeneic HCT following myeloablative conditioning**

The term "chimerism" has referred to the presence of lympho-hematopoietic cells of donor origin after an allogeneic HCT,\textsuperscript{18} and "full or complete chimerism" has been defined as complete replacement of host by donor lympho-hematopoiesis. For practical reason, mixed chimerism was defined as the detection of 5–95% cells of donor origin in hematopoietic tissues, which approximately defined the sensitivity of routinely used assays for quantifying chimerism.

Mixed chimerism was first observed in patients with advanced acute leukemia conditioned with cyclophosphamide alone.\textsuperscript{19} In patients with aplastic anemia conditioned with high doses of cyclophosphamide with or without anti-thymocyte globulin (ATG), mixed host/donor chimerism was found in a substantial proportion of patients, and this was associated with higher risk of graft rejection and, in the patients with sustained engraftment, a lower risk of acute GVHD.\textsuperscript{20,21} Branch et al.\textsuperscript{22} and Petz et al.\textsuperscript{23} reported mixed chimerism in some patients with hematologic malignancies transplanted with unmodified marrow grafts after myeloablative conditioning. In these studies, the presence of mixed chimerism did not predict subsequent disease relapse. However, Mackinnon et al.\textsuperscript{24} showed that minimal residual disease was more common in chronic myeloid leukemia (CML) patients with mixed chimerism, and Huss et al.\textsuperscript{21} reported that the presence of mixed chimerism after day 100 was associated with increased relapse risks in CML patients. In addition, mixed chimerism has been found frequently in patients who received T-cell-depleted grafts after myeloablative conditioning,\textsuperscript{25} indirectly implying that the recipients’ lympho-hematopoiesis was eradicated not only by the conditioning regimens but also by donor T cells.

**Methods for chimerism assessment**

Several methods have been employed to determine the degree of donor engraftment after allogeneic HCT, including conventional and molecular cyto genetics (for sex mismatched donor-recipient pairs or for patients with diseases that carry cytogenetic abnormalities), immunoglobulin allotypes, erythrocyte cell antigens, leukocyte isoenzymes, fluorescence in situ hybridization (FISH), and variable number tandem repeat (VNTR) polymorphism analyses (Table 2).\textsuperscript{18} As for the latter, certain core DNA sequences are tandemly dispersed and repeated throughout the genome, and the number of the tandem repeats of the core sequences can vary between individuals. Repeats can be composed of "microsatellite" (also called short tandem repeats or STR) sequences of 2–8 bp in length and repeated up to 100 times,\textsuperscript{26,27} or of "minisatellites" sequences of 8–50 bp in length.\textsuperscript{28,29} These repeated core sequences within a locus are characterized by extensive polymorphism and Mendelian codominant inheritance.\textsuperscript{30,31} Polymorphic microsatellite and minisatellite markers have advantages over techniques that detect sex chromosomes in that they can be used for virtually all donor-recipient pairs and, when used in combination with DNA amplification by polymerase chain reaction (PCR) techniques, only small numbers of cells are required for the test. As a result, microsatellites and minisatellites have been used extensively as markers of engraftment and for evaluation of the degree of chimerism in marrow transplantation in dogs and humans.\textsuperscript{32–34} For quantification, PCR products have been electrophoresed on an agarose gel, hybridized with \textsuperscript{32P}-labeled probes, autoradiographed and quantified by phosphor imaging (Fig. 1) or PCR was carried out with fluorescently labeled primers and the PCR product visualized using the ABI 310 sequencer (Applied Biosystems, Foster City, CA). Depending on fragment length and efficiency of amplification, the sensitivity of these assays is between 0.1% and 5%.\textsuperscript{18,35}

Short insertion/deletion polymorphisms detectable by quantitative real-time PCR have been introduced as alternatives to VNTR or STR markers for monitoring chimerism levels after HCT.\textsuperscript{36} Single-nucleotide polymorphisms (SNPs) that occur on average at every 1.3 kb in the human genome are thought to be the most common type of genetic variation, and the use of a multiplex microarray-
<table>
<thead>
<tr>
<th>Assay (reference)</th>
<th>Probability of two-way informative markers with sibling pairs (%)</th>
<th>Assay sensitivity (%)</th>
<th>Quantitative accuracy</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Erythrocyte antigens(^{53})</td>
<td>75–80</td>
<td>0.1–0.5</td>
<td>Moderate</td>
<td>• Studies limited to the erythroid lineage</td>
</tr>
<tr>
<td>Isoenzymes(^{53})</td>
<td>95</td>
<td>10–30</td>
<td>Moderate</td>
<td>• Low sensitivity</td>
</tr>
<tr>
<td>Conventional cytogenetics(^{53})</td>
<td>50</td>
<td>10–20</td>
<td>Low</td>
<td>• Low sensitivity</td>
</tr>
<tr>
<td>FISH(^{53})</td>
<td>50</td>
<td>1–2</td>
<td>High</td>
<td>• Only available for sex-mismatch HCT (or when an informative autosomal marker is present)</td>
</tr>
<tr>
<td>RFLP(^{53})</td>
<td>97</td>
<td>10–20</td>
<td>Moderate</td>
<td>• Technical difficulty</td>
</tr>
<tr>
<td>VNTR/STR(^{53})</td>
<td>90–100</td>
<td>5–10</td>
<td>Low</td>
<td>• Low quantitative accuracy</td>
</tr>
<tr>
<td>VNTR/STR with phosphorimaging(^{54})</td>
<td>90–100</td>
<td>0.1–1</td>
<td>Moderate</td>
<td>• Radioactivity</td>
</tr>
<tr>
<td>Multiplex STR amplification and fluorescence detection(^{55})</td>
<td>90–100</td>
<td>1–5</td>
<td>High</td>
<td>–</td>
</tr>
<tr>
<td>Real-time PCR STR amplification(^{56})</td>
<td>90–100</td>
<td>0.1–1</td>
<td>High</td>
<td>–</td>
</tr>
<tr>
<td>Multiplex SNP genotyping(^{36})</td>
<td>99–100</td>
<td>1</td>
<td>High</td>
<td>• Technical difficulty</td>
</tr>
</tbody>
</table>
Based minisequencing system screening 51 SNPs was recently shown to provide accurate chimerism quantification.

### Engraftment after nonmyeloablative or reduced-intensity conditioning regimens

#### Kinetics of engraftment

With the exception of cyclophosphamide-conditioned patients with aplastic anemia, engraftment after nonmyeloablative conditioning regimen was first analyzed by Childs et al. in 15 patients conditioned with cyclophosphamide and fludarabine and given postgrafting immunosuppression with CSP. The patterns of engraftment varied considerably but most often full donor chimerism was achieved earlier in T cells than in granulocytes, and the achievement of full donor T-cell chimerism preceded acute GVHD and anti-tumor responses. The kinetics of B-cell recovery were distinct from those of myeloid and T-cell lineages, while natural killer (NK) cell chimerism was closely correlated with T-cell chimerism.

Ueno et al. studied chimerism evolution in 23 patients with metastatic tumors transplanted after a reduced-intensity conditioning regimen consisting of fludarabine and melphalan. Postgrafting immunosuppression included tacrolimus and short methotrexate. All patients showed 100% T-cell and granulocyte chimerisms on days 30 and 100 after the transplant.

Dey et al. analyzed engraftment kinetics in 42 patients with advanced hematologic malignancies receiving allogeneic HCT after a regimen consisting of cyclophosphamide, anti-thymocyte globulin and thymic irradiation (the latter was given only to those patients who had not received previous mediastinal irradiation). Postgrafting immunosuppression consisted of a short course of CSP. On day 30, median T-cell chimerism was 50% (range, 1–95%). Nineteen evaluable patients subsequently achieved full donor T-cell chimerism, and 14 rejected their transplant by day 100 after HCT.

We analyzed the kinetics of donor engraftment in various peripheral blood cell subpopulations and their relation to HCT outcomes in a cohort of 120 patients given grafts from HLA-matched related or unrelated donors after conditioning consisting of 2 Gy TBI±fludarabine, 30 mg/m²/day. Postgrafting immunosuppression included MMF plus CSP (Fig. 2). While most patients rapidly developed high degrees of donor engraftment, they remained mixed donor/host chimeras for up to 6 months after HCT (Fig. 3). Generally, donor T-cell...
chimerism lagged behind myeloid chimerism. Donor T-cell chimerism on day 14 and 28 after HCT correlated closely with both donor CD4+ ($R = 0.94$) and CD8+ T-cell subset ($R = 0.90$) chimerism levels. Correlations between donor T-cell content and those among granulocytes ($R = 0.37$), NK cells ($R = 0.66$) and monocytes ($R = 0.56$) were weaker at the same time points.

Associations between transplant variables and chimerism levels

In addition to the intensity of the conditioning regimen, several other variables have been associated with chimerism levels after allogeneic HCT. These variables either influenced the recipients’ immune competence, thereby altering host-versus-graft reactions, or the donor T-cells and, therewith, graft-versus-host reactions.

Variables affecting host-versus-graft reactions

Previous chemotherapy

Several reports have shown relationships between previous chemotherapy exposure and chimerism levels. Valcarcel et al. studied 68 patients transplanted after conditioning with fludarabine (30 mg/m² on days $−8$ to $−4$) and melphalan (70 mg/m² on days $−3$ and $−2$) in patients with lymphoid malignancies or fludarabine (30 mg/m² on days $−9$ to $−5$) and busulfan (total 10 mg/kg) in patients with myeloid malignancies. GVHD prophylaxis consisted of CSP and a short course of methotrexate (MTX). In multivariate analysis, having received more than two lines of chemotherapy pretransplant was significantly associated with complete donor chimerism on day 30 after HCT among unfractionated nucleated peripheral blood cells.

Carvallo et al. analyzed pre-transplant variables affecting chimerism levels in 36 patients with metastatic solid tumors conditioned with fludarabine/busulfan. Postgrafting immunosuppression consisted of CSP alone or CSP combined with either MMF or MTX. At day 30, median T-cell and granulocyte chimerism levels were 98% and 76%, respectively, in patients who had prior chemotherapy versus 88% ($p = 0.008$) and 26% ($p < 0.0001$), respectively, in patients who had not.

In our study, a univariate analysis of data from patients who had received intensive chemotherapy before HCT showed higher donor T-cell, granulocyte, and monocyte chimerism levels ($p=0.002$, $0.002$ and $0.01$, respectively), compared to those who did not. There was also a trend towards higher donor NK cell chimerism ($p=0.10$). In multivariate analysis, intensive chemotherapy before HCT was associated with higher T-cell ($p=0.002$, average 21% increase compared to no chemotherapy) and monocyte ($p=0.04$, average 15% increase compared to no chemotherapy) chimerism levels.

Hematologic disease category

We also analyzed the impact of underlying hematologic diseases on engraftment kinetics. Patients with MDS and CML had lower levels of T-cell chimerisms than patients with AML or with lymphoid malignancies ($p=0.03$). After adjusting for intensive previous chemotherapy, the impact on disease category on T-cell chimerism levels was no longer significant.

Variables affecting graft-versus-host reactions

Stem cell source/graft composition

Patients who received marrow as stem cell source had lower percentages of donor T-cell chimerism ($p=0.002$) and a trend to lower donor NK-cell chimerism ($p=0.10$) than patients who received G-CSF-mobilized peripheral blood mononuclear cells (G-PBMC) (Fig. 4).

Carvallo et al. found that CD34+ graft content positively correlated with the degree of donor myeloid chimerism, but failed to establish a correlation between graft composition and donor T-cell chimerism levels.

Baron et al. analyzed T-cell chimerism in 35 patients conditioned with TBI (2 Gy) alone ($N=15$), TBI (2 Gy) and fludarabine ($N=13$), or fludarabine
and cyclophosphamide (N=7). Patients received either unmanipulated- (N=18), CD8-depleted- (N=11) or CD34-selected-G-PBMC (N=6). Postgrafting immunosuppression included MMF and CSP. Median donor T-cell contributions on days 28, 60, 100, 180 and 365 in recipients of unmanipulated G-PBMC were 75%, 85%, 87%, 90% and 100%, respectively. Evolution of donor T-cell chimerism did not differ significantly between recipients of unmanipulated versus CD8-depleted G-PBMC while CD34 selection resulted in significantly decreased donor T-cell chimerism42 (Fig. 5).

We also found significant correlations between graft contents and levels of donor chimerisms.39 Higher T-cell (p=0.003), NK cell (p=0.03) and monocyte (p=0.0002) contents in the graft were associated with higher T-cell donor chimerisms, while higher CD34+ cell (p=0.002) and monocyte (p=0.04) contents resulted in higher monocyte donor chimerism. In multivariate analysis, higher numbers of monocytes in the graft (p=0.005, modeled as a continuous linear variable) were associated with increasing donor T-cell chimerism.

Donor type
We found no differences in chimerism levels among patients who received HLA-matched related compared to unrelated G-PBMC.39

Associations between chimerism levels and HCT outcomes after reduced-intensity or nonmyeloablative conditioning

Graft rejection
An increased incidence of graft rejection in aplastic anemia patients with mixed donor/host chimerism after conditioning with cyclophosphamide was first reported in 1986.20 Few studies to date have analyzed the impact of lineage-specific chimerism levels on graft rejection. Bornhauser et al.43 suggested that fludarabine/busulfan-conditioned patients with NK-cell donor chimerism levels below 75% on days 10–30 after HCT were more likely to have graft failure than those with more than 75% (p=0.03). However, NK-cell chimerism levels were available in only 10 patients. Matthes-Martin et al.44 showed that day 28 T-cell (p=0.001) and NK-cell (p=0.0001) chimerism levels were strongly correlated with late graft rejection in pediatric patients conditioned with a reduced-intensity regimen. In contrast, granulocyte/monocyte chimerism was less reliable in predicting graft rejection.

In our study, both day-14 NK- and T-cell chimerism levels <50% were associated with significantly increased risks of subsequent graft rejection (p=0.01 and p=0.02, respectively, after adjusting for donor type (related versus unrelated)) (Table 3).39

Graft-versus-host disease
A decreased incidence of acute GVHD in patients with sustained engraftment and mixed donor/host chimerism was first reported in aplastic anemia patients conditioned with cyclophosphamide.20 More recently, Childs et al.14 reported that achievement of complete donor T-cell chimerism always preceded grade 2–4 acute GVHD. However, Mattson

Figure 4 Kinetics of donor T-cell engraftment in patients receiving G-CSF mobilized peripheral blood mononuclear cells (G-PBMC, n=110) or bone marrow (marrow, n=10) as stem cell sources.

Figure 5 Kinetics of donor T-cell engraftment in patients receiving unmanipulated (n=18) or CD34-selected (n=6) G-CSF mobilized peripheral blood mononuclear cells (G-PBMC) as stem cell sources after nonmyeloablative regimens consisting of TBI alone (n=10), TBI+fludarabine (n=10) or cyclophosphamide plus fludarabine (n=4). Adapted from Baron F, Schaaf-Lafontaine N, Humblet-Baron S, et al. T-cell reconstitution after unmanipulated, CD8-depleted or CD34-selected nonmyeloablative peripheral blood stem-cell transplantation. Transplantation 2003;76:1705–13 [reference 42]. Used with permission.
et al. found that 90% of their patients (conditioned with four nonmyeloablative regimens, including fludarabine, busulfan, TBI, cyclophosphamide and ATG) had mixed donor/host T-cell chimerism at the onset of acute GVHD.

Petersen et al. \(^4\) proposed that donor CD8\(^+\) T-cell count above the median (0.043 \( \times \) 10\(^6\) cells/\( \mu l \)) on day 14 after HCT predicted the risk of subsequent development of grade 2–4 acute GVHD. However, only 24 patients were included in that study.

We showed that, with increasing levels of donor T-cell chimerisms on day 28, the probability of subsequent grade 2–4 acute GVHD increased. The risk of subsequent development of acute GVHD was 27% in patients with T-cell chimerism levels of \( \leq 50\% \), but was 75% in patients with >90% T-cell chimerism levels \( (p = 0.02 \text{ after adjusting for donor type}) \). As observed by Mattson et al., most patients with grades 2 and 3–4 acute GVHD were mixed donor/host chimeras at onset of GVHD.

Graft-versus-tumor effect/relapse

Two recent reports suggested a relationship between chimerism levels and disease responses. Childs et al. reported that achievement of 100% donor T-cell engraftment always preceded anti-tumor responses. Perez-Simon et al. found a trend for a higher relapse risk in patients with mixed T-cell chimerism as compared to patients with complete donor chimerism after conditioning with fludarabine/melphalan or fludarabine/busulfan.

In our study, 93 of 120 patients had measurable malignant disease before transplantation, and 41 of the 93 (44%) achieved complete remissions 199 (range, 28–963) days after HCT. At the time of achievement of complete remissions, 19 of the 41 patients showed mixed donor/host T-cell chimerism and 22 had complete donor T-cell chimerism. Neither day 28 T-cell nor NK-cell chimerism levels were significantly associated with disease responses, although there was a suggestion that patients with T-cell chimerism levels <50% were at higher risk of failing to achieve complete remissions (20% versus 47%, not significant).

Progression-free survival

Keil et al. \(^4\) found an improved progression-free survival in patients with >90% donor T-cell chimerism compared to those with <90% \( (p < 0.002) \) after conditioning with 2 Gy TBI plus fludarabine.

In our study, there were no correlations between early cell subset chimerism levels and progression-free survival. However, when chimerism data from days 14–100 were combined, higher donor NK cell chimerism levels were associated with statistically significantly improved progression-free survival \( \text{[HR 0.79, 95% CI (0.64–0.96), } p = 0.02] \).

Summary

Engraftment kinetics after nonmyeloablative or reduced-intensity conditioning depend on the intensity of pretransplant chemotherapy, the intensity of the conditioning regimens, the graft composition, and whether grafts have been depleted of T cells. Monitoring mixed chimerism among peripheral blood subpopulation early after transplant identified patients at risk for graft rejection, acute GVHD, and death/relapse, and this knowledge might allow early intervention with immunosuppressive drugs or DLI aimed at obviating these complications.

Practice points

- Kinetics of donor engraftment are different among T cells, NK cells, granulocytes and monocytes.

### Table 3

<table>
<thead>
<tr>
<th>% donor chimerism(^b,c) on day 14</th>
<th>% of patients with rejection (# pts at risk)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>T cells</td>
</tr>
<tr>
<td>0–50</td>
<td>25 (( n = 32 ))</td>
</tr>
<tr>
<td>51–75</td>
<td>2.5 (( n = 40 ))</td>
</tr>
<tr>
<td>76–90</td>
<td>0 (( n = 22 ))</td>
</tr>
<tr>
<td>91–100</td>
<td>0 (( n = 6 ))</td>
</tr>
</tbody>
</table>

\(^a\) \( p \) values obtained trend test from logistic regression model after adjusting for presence of an unrelated donor.

\(^b\) Day-14 T-cell, NK-cell, monocyte and granulocyte chimerism levels were available for 100, 86 and 96 patients, respectively.

\(^c\) Percent donor chimerism as a categorical variable.
• Patients given intensive preceding therapy have higher granulocyte, T-cell and monocyte donor chimerism levels.
• G-PBMC as a stem cell source is associated with increased T-cell donor chimerism levels.
• Day-14 T- and NK-cell chimerism levels predicted patients at risk for subsequent graft rejection.
• Day-28 T-cell chimerism levels predicted patients at risk for subsequent GVHD.

Research agenda
• Engraftment kinetics of naive and memory T cells.
• Impact of CD4 and CD8 T-cell chimerism on subsequent risks of rejection/GVHD.
• Impact of chimerism levels on progression-free survival in a large group of patients with similar diseases.

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