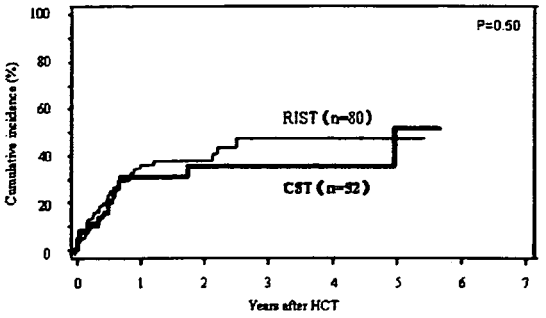


**Figure 2.** Cumulative incidence of NRM. The 2-year probabilities of NRM in the CST (36%) and RIST (38%) groups were not significantly different ($P$ = .50).

29%, $P$ = .89, for PFS), as shown in Figure 3 and Figure 4. The 2-year probabilities of PD, OS, and PFS were not significantly different between patients who developed grade III-IV aGVHD and those who did not (37% and 44%, $P$ = .39, for PD; 33% and 50%, $P$ = .07, for OS; 27% and 41%, $P$ = .24, for PFS). On the other hand, the 2-year probability of NRM in patients who developed grade III-IV aGVHD was significantly higher than that in those who did not (56% and 21%, $P$ = .004). We also evaluated outcomes in patients who had AML or MDS (CST, n = 35; RIST, n = 39). There was no significant difference in the 2-year probabilities of PD (50% and 51%), OS (37% and 33%), and PFS (34% and 22%) between the CST and RIST groups. On the other hand, the 2-year probability of NRM in the RIST group was significantly higher than that in the CST group (52% and 23%, $P$ = .03).

Multivariate analyses in all patients showed that a higher HCT-CI score (1 or more) and transplant from an alternative donor were associated with poor OS, and patients who received chemotherapy within 2 months before HCT were associated with poor OS and PFS (Table 4). After adjusting for these variables, the risks of OS and PFS were not significantly different between the CST and RIST groups. Disease type (leukemia/MDS or lymphoma) was not a significant factor for OS or PFS. Furthermore, subset analyses revealed that a higher HCT-CI score (1 or more) was associated with poor OS and PFS in the CST group, but not in the RIST group (Table 5). In contrast, transplant from an alternative donor was associated with increased NRM in the RIST group, but not in the CST group. Patients who received chemotherapy within 2 months before HCT had a poor PFS in both groups.

## DISCUSSION

Our results suggest that the antileukemia/lymphoma effect of RIST might be comparable to that of CST for hematologic malignancies that are not in remission. We found that a higher HCT-CI score and transplant from an alternative donor were associated with increased risks of NRM and poor OS, and patients who received chemotherapy within 2 months before HCT because of the acceleration of disease progression were associated with increased risks of PD, poor OS, and PFS. The estimated rates of NRM, PD, OS, and PFS in the RIST group were not significantly different from those in the CST group even though the patients who received RIST were significantly older and had significantly higher HCT-CI scores than those who received CST. Several reports have described a similar OS rate in older patients who underwent RIST and CST because the lower NRM rate was offset by a higher PD [5,27,28]. In contrast, Scott et al. [7] found no significant differences in OS, PFS, PD, or NRM between CST and RIST in patients with MDS/AML.

In this study, disease response to the transplantation procedure was similar between the CST and RIST groups when the CR rate is considered the best response, as were the rate and timing of PD. Whereas some reports have shown that PD after HCT was increased in patients who underwent RIST compared to CST [3,5,11], others have found no significant difference [6-8,29]. This discrepancy might result from the differences in disease status at the time of

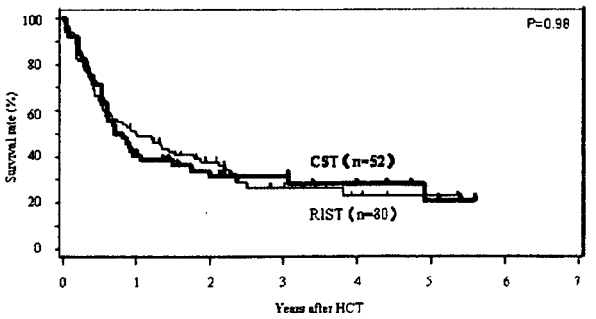


**Figure 3.** Estimated OS according to the conditioning regimen. The 2-year probabilities of OS in the CST (31%) and RIST (38%) groups were not significantly different ($P$ = .98).

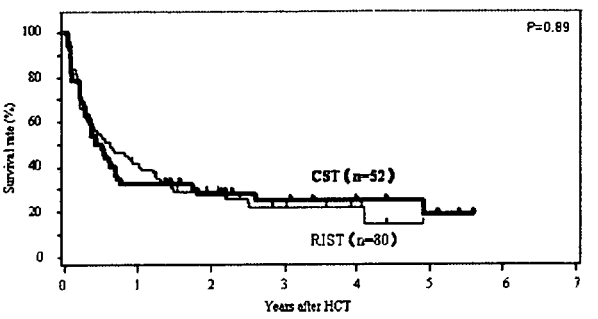


**Figure 4.** Estimated PFS according to the conditioning regimen. The 2-year probabilities of PFS in the CST (28%) and RIST (29%) groups were not significantly different ($P$ = .89).

transplantation and the intensity of the conditioning regimens. In our study, the median percentage of blasts in leukemia/MDS patients and the distribution of serum LDH levels in lymphoma patients were comparable between the CST and RIST groups. The proportion of patients who required chemotherapy within 2 months before HCT was similar in the 2 groups. Overall, the risk of disease progression was comparable. The lack of a significant difference in PD between the CST and RIST groups in our study may be because the reduced-intensity regimens used in our study were more intense than those in previous reports. Nevertheless, our results suggest that RIST has a comparable antileukemia/lymphoma activity through a GVL effect compared to CST.

Our study found that chemotherapy within 2 months before HCT was the only factor that significantly predicted a lower CR rate in lymphoma patients and tended to be associated with a lower CR rate in leukemia/MDS patients. Furthermore, chemotherapy within 2 months before HCT was also associated with a worse prognosis not only with regard to PD but also for OS and PFS. A subset analysis showed that this negative impact of recent chemotherapy was only seen in RIST patients, and not in CST patients, which suggests that the tempo of the progression of the disease before HCT is especially important in RIST patients. Wong et al. [30] reported that high peripheral blast counts (≥30%) in patients with AML/MDS were associated with poor event-free survival and OS after HCT regardless of the conditioning regimen. In our study, however, ≥20% of blasts in the bone marrow or peripheral blood and serum LDH level elevation did not have a significant impact on the CR rate in leukemia/MDS and lymphoma patients, respectively.

In our study, there was no significant difference in NRM between the CST and RIST groups, which was in contrast to previous reports showing that reduced-intensity regimens were associated with less organ damage, and thus contributed to less NRM [1,4,5,9,27,31-34]. There are several possible explanations for this discrepancy. First, the patients who received RIST were older and had a higher HCT-CI score than those in the CST group. Second, the reduced-intensity conditioning (RIC) we used was more toxic than "truly nonmyeloablative" conditioning. Finally, we tapered immunosuppressive medications rapidly, especially in the RIST group, in an attempt to induce a more potent GVL effect, which resulted in more severe GVHD and subsequent infectious complications. However, our data showed that grade III-IV aGVHD did not contribute to a reduction in the rate of PD or to an overall improvement in survival, which was consistent with a previous report [14], although a high rate of NRM in patients with severe aGVHD may have masked its competing event (ie, PD).

We confirmed that HCT-CI was a significant risk factor for NRM and OS in patients not in remission. HCT-CI has recently been introduced to evaluate pretransplant comorbidities in HCT recipients, which predict well NRM and OS after allogeneic HCT [20]. In this study, the proportion of patients who were not in remission and were associated with comorbidities was 53%, which was higher than the value (42%) in our previous report [21], probably because these patients tended to be heavily pretreated and were forced to pursue HCT in the hope of a rare cure. Interestingly, this negative impact of HCT-CI was only seen in patients who underwent CST, and not in those who underwent RIST. Our data imply that RIC may be preferable in patients with hematologic malignancies not in remission and with a high HCT-CI score by reducing early NRM after transplantation.

Transplant from an alternative donor was another prognostic factor for NRM and OS in this study, which is consistent with previous reports [12,35-38]. Furthermore, an increased risk of NRM and OS associated with alternative donors was observed only in patients who underwent RIST. There are several possible explanations. First, the Japan Marrow Donor Program allows the donation of bone marrow, but not PBSC, from volunteer donors, which has been reported to be associated with poor engraftment and worse outcomes after nonmyeloablative stem cell transplantation [13]. Second, our conditioning regimen including low-dose TBI for RIST from an alternative donor was more toxic than that for RIST from an HLA-matched related donor. Further studies are required to establish optimized conditioning regimens and GVHD prophylaxis for RIST in unrelated pair settings.

In 27 patients who had all of these risk factors (ie, chemotherapy within 2 months beore HCT, HCT-CI score of 1 or more, and transplant from an alternative donor), the 2-year probabilities of NRM, PD, and OS were 56%, 44%, and 21%, respectively, with no significant differences between the CST and RIST groups (data not shown). Therefore, the indications for transplantation in patients with multiple risk factors should be carefully determined.

This study has several inherent limitations. First, the eligibility requirements for CST and RIST were different. Most patients who received RIST were considered ineligible for CST because of age or comorbid conditions. Second, factors other than the conditioning regimen were not entirely comparable between the 2 groups, that is, patient age, underlying diagnosis (leukemia/MDS and lymphoma), donor selection, stem cell source, and GVHD prophylaxis. Third, some of the conventional cytoreductive conditioning regimens we used (ie, use of oral BU and lack of its pharmacologic monitoring) may no longer be considered optimal. Fourth, because the reduced-intensity

regimens used in our study were more intense than those in previous reports, our data may not be generalized to the concept of "reduced-intensity regimen" and there may be circumstances where PD would be more marked. Finally, the follow-up of patients in this study was too short to draw any definite conclusions. Nevertheless, the observed data may still be useful in evaluating the impact of RIST on disease control in patients suffering from a higher risk of disease progression after transplantation.

In conclusion, our results suggest that the antileukemia/lymphoma effect associated with RIST might be comparable to that of CST for hematologic malignancies not in remission, particularly when patients do not require chemotherapy within 2 months before HCT or they had a higher HCT-CI score. To determine the ultimate utility of specific conditioning regimens, controlled prospective trials are needed, with enrolled patients being stratified according to disease activity, hematopoietic stem cell source, and associated comorbidities.

## ACKNOWLEDGMENTS

This work was supported in part by grants from the Ministry of Health, Labor and Welfare, Japan. This paper was presented in part as a poster presentation at the 47th Annual Meeting of the American Society of Hematology, Atlanta, GA, December 2005. We thank the medical, nursing, data processing, and laboratory staffs at National Cancer Center Hospital for their important contributions to this study through dedicated care of the patients. We also thank Yukio Kobayashi, Takashi Watanabe, Fusako Ohara, and Kazuaki Yokoyama for their helpful discussions.

## REFERENCES

1. McSweeney PA, Niederwieser D, Shizuru JA, et al. Hematopoietic cell transplantation in older patients with hematologic malignancies: replacing high-dose cytotoxic therapy with graft-versus-tumor effects. *Blood.* 2001;97:3390-3400.
2. Bertz H, Potthoff K, Finke J. Allogeneic stem-cell transplantation from related and unrelated donors in older patients with myeloid leukemia. *J Clin Oncol.* 2003;21:1480-1484.
3. Giralt S, Estey E, Albitar M, et al. Engraftment of allogeneic hematopoietic progenitor cells with purine analog-containing chemotherapy: harnessing graft-versus-leukemia without myeloablative therapy. *Blood.* 1997;89:4531-4536.
4. Slavin S, Nagler A, Naparstek E, et al. Nonmyeloablative stem cell transplantation and cell therapy as an alternative to conventional bone marrow transplantation with lethal cytoreduction for the treatment of malignant and nonmalignant hematologic diseases. *Blood.* 1998;91:756-763.
5. Alyea EP, Haesook T, Soiffer RJ, et al. Comparative outcome of nonmyeloablative and myeloablative allogeneic hematopoietic cell transplantation for patients older than 50 years of age. *Blood.* 2005;105:1810-1814.
6. Valcá rcel D, Martino R, Sierra J, et al. Conventional versus reduced-intensity conditioning regimen for allogeneic stem cell transplantation in patients with hematological malignancies. *Eur J Haematol.* 2005;74:144-151.
7. Scott BL, Sandmaier BM, Storer B, et al. Myeloablative vs nonmyeloablative allogeneic transplantation for patients with myelodysplastic syndrome or acute myelogenous leukemia with multilineage dysplasia: a retrospective analysis. *Leukemia.* 2006; 20:128-135.
8. Kojima R, Kami M, Kanda Y, et al. Comparison between reduced intensity and conventional myeloablative allogeneic stem-cell transplantation in patients with hematologic malignancies aged between 50 and 59 years. *Bone Marrow Transplant.* 2005;36:667-674.
9. Mirlcarek M, Martin PJ, Leisenring W, et al. Graft-versus-host disease after nonmyeloablative versus conventional hematopoietic stem cell transplantation. *Blood.* 2003;102:756-762.
10. Nagler A, Slavin S, Varadi G et al. Allogeneic peripheral blood stem cell transplantation using a fludarabine-based low intensity conditioning regimen for malignant lymphoma. *Bone Marrow Transplant.* 2000;25:1021-1028.
11. Michallet M, Bilger K, Garban F, et al. Allogeneic hematopoietic stem-cell transplantation after nonmyeloablative preparative regimens: impact of pretransplantation and posttransplantation factors on outcome. *J Clin Oncol.* 2001;19:3340-3349.
12. Baron F, Storb R, Storer BE, et al. Factors associated with outcomes in allogeneic hematopoietic cell transplantation with nonmyeloablative conditioning after failed myeloablative hematopoietic cell transplantation. *J Clin Oncol.* 2006;24:4150-4157.
13. Maris MB, Niederwiwser D, Sandmaier BM, et al. HLA-matched unrelated donor hematopoietic cell transplantation after nonmyeloablative conditioning for patients with hematologic malignancies. *Blood.* 2003;102:2021-2030.
14. Baron F, Maris MB, Sandmaier BM, et al. Graft-versus-tumor effects after allogeneic hematopoietic cell transplantation with nonmyeloablative conditioning. *J Clin Oncol.* 2005;23:1993-2003.
15. Saito T, Kanda Y, Kami M, et al. Therapeutic potential of a reduced-intensity preparative regimen for allogeneic transplantation with cladribine, busulfan, and antithymocyte globulin against advanced/refractory acute leukemia/lymphoma. *Clin Cancer Res.* 2002;8:1014-1020.
16. Storb R, Deeg HJ, Whitehead J, et al. Methotrexate and cyclosporine compared with cyclosporine alone for prophylaxis of acute graft versus host disease after marrow transplantation for leukemia. *N Engl J Med.* 1986;314:729-735.
17. Kanda Y, Mineishi S, Saito T, et al. Long-term low-dose acyclovir against varicella-zoster virus reactivation after allogeneic hematopoietic stem cell transplantation. *Bone Marrow Transplant.* 2001;28:689-692.
18. Kanda Y, Mineishi S, Saito T, et al. Response-oriented preemptive therapy against cytomegalovirus disease with low-dose ganciclovir: a prospective evaluation. *Transplantation.* 2002;73: 568-572.
19. Junghanss C, Boeckh M, Carter RA, et al. Incidence and outcome of cytomegalovirus infections following nonmyeloablative compared with myeloablative allogeneic stem cell transplantation, a matched control study. *Blood.* 2002;99:1978-1985.
20. Sorror ML, Maris MB, Storner B, et al. Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. *Blood.* 2005;106:2912-2919.

21. Kato R, Fukuda T, Usui E, et al. Hematopoietic cell transplantation-specific comorbidity index to predict non-relapse mortality and survival after allografting. *Blood.* 2005;106:766a.
22. Glucksberg H, Storb R, Fefer A, et al. Clinical manifestations of graft-versus-host disease in human recipients of marrow from HL-A-matched sibling donors. *Transplantation.* 1974;18: 295-304.
23. Przepiorka D, Weisdorf D, Martin P, et al. Consensus conference on acute GVHD grading. *Bone Marrow Transplant.* 1995; 15:825-828.
24. Sullivan KM, Agura E, Anasetti C, et al. Chronic graft-versus-host disease and other late complications of bone marrow transplantation. *Semin Hematol.* 1991;28:250-259.
25. Cheson BD, Horning SJ, Coiffier B, et al. Report of an international workshop to standardize response criteria for non-Hodgkin's lymphoma. *J Clin Oncol.* 1999;17:1244-1253.
26. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. *J Am Stat Assoc.* 1958;53:457-481.
27. de Lima M, Anagnostopoulos A, Munsell, et al. Nonablative versus reduced-intensity conditioning regimens in the treatment of acute myeloid leukemia and high-risk myelodysplastic syndrome: dose is relevant for long-term disease control after allogeneic hematopoietic stem cell transplantation. *Blood.* 2004; 104:865-872.
28. Martino R, Iacobelli S, de Witte T, et al. Retrospective comparison of reduced-intensity conditioning and conventional high-dose conditioning for allogeneic hematopoietic stem cell transplantation using HLA-identical sibling donors in myelodysplastic syndromes. *Blood.* 2006;108:836-846.
29. Kim DH, Sohn SK, Baek JH, et al. Retrospective multicenter study of allogeneic peripheral blood stem cell transplantation followed by reduced-intensity conditioning or conventional myeloablative regimen. *Acta Haematol.* 2005;113:220-227.
30. Wong R, Shahjahan M, Wang X, et al. Prognostic factors for outcomes of patients with refractory or relapsed acute myelogenous leukemia or myelodysplastic syndromes undergoing allogeneic progenitor cell transplantation. *Biol Blood Marrow Transplant.* 2005;11:108-114.
31. Fukuda T, Hackman RC, Guthrie KA, et al. Risks and outcomes of idiopathic pneumonia syndrome after non-myeloablative and conventional conditioning regimens for allogeneic hematopoietic stem cell transplantation. *Blood.* 2003;102:2777-2785.
32. Sorror ML, Maris MB, Storer B, et al. Comparing morbidity and mortality of HLA-matched unrelated donor hematopoietic cell transplantation after nonmyeloablative and myeloablative conditioning: influence of pretransplant comorbidities. *Blood.* 2004;104:961-968.
33. Canals C, Martino R, Sureda A, et al. Strategies to reduce transplant-related mortality after allogeneic stem cell transplantation in elderly patients: comparison of reduced-intensity conditioning and unmanipulated peripheral blood stem cells vs a myeloablative regimen and CD34+ cell selection. *Exp Hematol.* 2003;31:1039-1043.
34. Diaconescu R, Flowers CR, Storer B, et al. Morbidity and mortality with nonmyeloablative compared to myeloablative conditioning before hematopoietic cell transplantation from HLA matched related donors. *Blood.* 2004;104:1550-1558.
35. Sasazuki T, Juji T, Morishima Y, et al. Effect of matching of class I HLA alleles on clinical outcome after transplantation of hematopoietic stem cells from an unrelated donor. *N Engl J Med.* 1998;339:1177-1185.
36. Junghanss C, Storb R, Maris MB, et al. Impact of unrelated donor status on the incidence and outcome of cytomegalovirus infections after non-myeloablative allogeneic stem cell transplantation. *Br J Haematol.* 2003;123:662-670.
37. Kanda Y, Chiba S, Hirai H, et al. Allogeneic hematopoietic stem cell transplantation from family members other than HLA-identical siblings over the last decade (1991-2000). *Blood.* 2003;102:1541-1547.
38. Arnold R, De Witte T, van Biezen A, et al. Unrelated bone marrow transplantation in patients with myelodysplastic syndromes and secondary acute myeloid leukemia: an EBMT survey. European Blood and Marrow Transplantation Group. *Bone Marrow Transplant.* 1998;21:1213-1216.

# Hyperglycemia During the Neutropenic Period Is Associated With a Poor Outcome in Patients Undergoing Myeloablative Allogeneic Hematopoietic Stem Cell Transplantation

*Shigeo Fuji,[1] Sung-Won Kim,[1] Shin-ichiro Mori,[1] Takahiro Fukuda,[1] Shigemi Kamiya,[2] Satoshi Yamasaki,[1] Yuriko Morita-Hoshi,[1] Fusako Ohara-Waki,[1] Osamu Honda,[3] Setsuko Kuwahara,[2] Ryuji Tanosaki,[1] Yuji Heike,[1] Kensei Tobinai,[1] and Yoichi Takaue[1,4]*

**Background.** Recipients of allogeneic hematopoietic stem cell transplantation (HSCT) frequently require support with parenteral nutrition and immunosuppressive drugs, which introduce the risk of hyperglycemia. Van den Berghe et al. showed that the strict glucose control improved the outcome of patients treated in the intensive care unit, and this point was evaluated in this study in a HSCT setting.

**Methods.** A cohort of 112 consecutive adult patients treated by myeloablative allogeneic HSCT between January 2002 and June 2006 was reviewed retrospectively. Twenty-one patients were excluded due to graft failure, preexisting infectious diseases, preexisting neutropenia or previous allogeneic HSCT. The remaining 91 patients were categorized according to mean fasting blood glucose (BG) level in the neutropenic period after conditioning: normoglycemia (BG <110 mg/dL, n=28), mild hyperglycemia (110 to 150 mg/dL, n=49), and moderate/severe (>150 mg/dL, n=14). The primary endpoint was the occurrence of febrile neutropenia (FN) and documented infection during neutropenia, and the secondary endpoints included organ dysfunction according to the definition used by van den Berghe, acute graft-versus-host disease (GVHD), overall survival, and nonrelapse mortality (NRM).

**Results.** Although the incidence of FN or documented infections was similar between the three groups, hyperglycemia was significantly associated with an increased risk of organ dysfunction, grade II–IV acute GVHD, and NRM.

**Conclusions.** While the results suggested an association between the degree of hyperglycemia during neutropenia and an increased risk of posttransplant complications and NRM, the possibility that intensive glucose control improves the outcome after HSCT can only be confirmed in a prospective randomized trial.

**Keywords:** Allogeneic transplantation, Hyperglycemia, Nonrelapse mortality, Acute graft-versus-host disease.

(*Transplantation* 2007;84: 814–820)

Van den Berghe et al. showed with patients nursed in the intensive care unit (ICU) that the rigid control of hyperglycemia with intensive insulin therapy to keep the blood glucose level at 80–110 mg/dL reduced morbidity, including infections, and mortality compared to patients who received standard care maneuvers that maintained the level at <200 mg/dL (*1–3*). Although these results have been confirmed in several subsequent studies (*4–7*), the precise mechanism that underlies this association is unclear. In animal models, it has been shown that insulin itself has a direct inhibitory effect on the inflammation process (*8, 9*). However in human studies, it has been suggested that these benefits could be directly attributed to intense glucose control rather than to any pharmacological activity of administered insulin per se (*3, 4*).

Recipients of allogeneic hematopoietic stem cell transplantation (HSCT) suffer from serious complications including infection, graft-versus-host disease (GVHD) and organ dysfunction. They are also at higher risk of hyperglycemia due to the use of steroids for the treatment of graft-versus-host disease (GVHD), prolonged total parenteral nutrition (TPN), immunosuppressive drugs, and infectious complications (*10, 11*). This makes them susceptible to numerous serious complications, including multiple organ failure (*12–14*). In this study, we evaluated whether hyperglycemia during the cytopenic pe-

Supported in part by grants from the Ministry of Health, Labor and Welfare, Japan.

This paper was presented in part as a poster presentation at the tandem Meeting of ASBMT and IBMTR, Keystone, Colorado, February 2007.

[1] Department of Hematology and Stem Cell Transplantation, National Cancer Center Hospital, Tokyo, Japan.

[2] Division of Nutritional Management, National Cancer Center Hospital, Tokyo, Japan.

[3] Tokyo Anesthesiology Group, Tokyo, Japan.

[4] Address correspondence to: Yoichi Takaue, M.D., Department of Hematology, National Cancer Center Hospital., 5-1-1, Tsukiji, Chuo-Ku, Tokyo 104-0045, Japan.

E-mail: ytakaue@ncc.go.jp

Received 12 June 2007. Revision requested 10 July 2007.

Accepted 18 July 2007.



ISSN 0041-1337/07/8407-814

DOI: 10.1097/01.tp.0000282790.66889.a5

riod after conditioning for HSCT could be a significant risk factor for the subsequent clinical course.

## PATIENTS AND METHODS

### Patient Characteristics

A cohort of 112 consecutive adult patients who received myeloablative allogeneic HSCT between January 2002 and June 2006 at the National Cancer Center Hospital (Tokyo, Japan) was reviewed retrospectively. Twenty-one patients were excluded due to graft failure, pre-existing infectious diseases or neutropenia before HSCT, and previous allogeneic HSCT. The remaining 91 patients were subjected to further analysis, and their characteristics are listed in Table 1. Their median age was 36 years (range, 18–57 years), and their diagnosis included acute myeloid leukemia (AML, n=41), acute lymphoblastic leukemia (ALL, n=21), non-Hodgkin lymphoma (NHL, n=13), myelodysplastic syndrome (MDS, n=10), and chronic myelogenous leukemia (n=6). Standard-risk patients included those with acute leukemia in first complete remission, chronic leukemia in first chronic phase, MDS in refractory anemia, and NHL in complete remission, and the remaining patients were categorized as high-risk. Forty-six and 45 patients received a graft from a related donor and an unrelated donor, respectively. Stem cell sources included bone marrow (n=46), peripheral blood (n=41), and cord blood cells (n=4). In this study, only two patients were diagnosed as type 2 diabetes mellitus before HSCT, which reflects the low prevalence of this condition in Japan, especially in younger patients who can be the target of allogeneic HSCT with a myeloablative conditioning regimen. These two diabetic patients were included in the moderate and severe hyperglycemia group. None of the patients, including these two patients, had major organ dysfunction or diabetic complications before HSCT. For the transplantation procedure, signed informed consent was obtained according to the Declaration of Helsinki.

### Transplantation Procedures

All patients received a myeloablative conditioning regimen that included oral busulfan (BU) plus cyclophosphamide (CY, n=45), CY plus 12 Gy total body irradiation (TBI, n=43) or cytarabine (CA) plus CY plus TBI (n=3: Table 1). GVHD prophylaxis included cyclosporine- (n=62) and tacrolimus-based regimens (n=29), with an additional short course of methotrexate (MTX) in 89 patients. Granulocyte

**TABLE 1.** Patient characteristics

| Variable | Normoglycemia (<110 mg/dl) | Mild hyperglycemia (110–150 mg/dl) | Moderate and severe hyperglycemia (>150 mg/dl) |
|---|---|---|---|
| N | 28 | 49 | 14 |
| Blood glucose, median mg/dl (range) | 104 (81–109) | 120 (110–150) | 168 (150–211) |
| Age, median years (range) | 31 (21–52) | 36 (18–57) | 45 (30–57) |
| <40 | 20 (71) | 32 (65) | 4 (29) |
| ≥40 | 8 (29) | 17 (35) | 10 (71) |
| Sex | | | |
| Male | 9 (32) | 34 (69) | 8 (57) |
| Female | 19 (68) | 15 (31) | 6 (43) |
| Disease risk | | | |
| Standard | 16 (57) | 18 (37) | 6 (43) |
| High | 12 (43) | 31 (63) | 8 (57) |
| Conditioning | | | |
| TBI-containing | 11 (39) | 26 (53) | 9 (64) |
| Non-TBI-containing | 17 (61) | 23 (47) | 5 (36) |
| GVHD prophylaxis | | | |
| Cyclosporine-based | 24 (86) | 33 (67) | 5 (36) |
| Tacrolimus-based | 4 (14) | 16 (33) | 9 (74) |
| Relation to donor | | | |
| Related | 19 (68) | 24 (49) | 3 (21) |
| Unrelated | 9 (32) | 25 (51) | 11 (79) |
| Stem cell source | | | |
| Bone marrow | 11 (39) | 24 (49) | 11 (79) |
| PBSC | 16 (57) | 22 (45) | 3 (21) |
| Cord blood | 1 (4) | 3 (6) | 0 (0) |
| HLA match | | | |
| Match | 25 (89) | 34 (69) | 10 (71) |
| Mismatch | 3 (11) | 15 (31) | 4 (29) |

Data are n (%) unless noted.
TBI, total body irradiation; GVHD, graft-versus-host disease; PBSC, peripheral blood stem cells; HLA, human leukocyte antigen.

colony-stimulating factor (G-CSF) was administered in all patients from day +6 after transplantation until engraftment. Most patients received ciprofloxacin (200 mg orally three times daily) for bacterial prophylaxis until neutrophil engraftment. Fluconazole (100 mg once daily) was administered for fungal prophylaxis. Low-dose acyclovir was given for prophylaxis against herpes simplex virus and varicella zoster virus until the cessation of immunosuppressive agents. Prophylaxis against *Pneumocystis jiroveci* infection consisted of trimethoprim-sulfamethoxazole (400 mg of sulfamethoxazole once daily) from the first day of conditioning to day −3 of transplantation, and from day +28 until day +180 or the cessation of immunosuppressive agents. Patients who developed fever during the neutropenic period were treated with cefepime, and additional agents including vancomycin, aminoglycosides and amphotericin B were given as clinically indicated. Neutrophil engraftment was defined as the first of 3 consecutive days after transplantation that the absolute neutrophil count exceeded $0.5\times10^9$/L.

### Grouping of Patients

Patients were categorized according to the mean blood glucose (BG) level in the preengraftment neutropenic period: normoglycemia BG maintained at <110 mg/dL (group 1, n=28), mild hyperglycemia at 110–150 mg/dL (group 2, n=49), and moderate/severe hyperglycemia at >150 mg/dL (group 3, n=14). Blood glucose level was routinely tested in the morning at least three times a week. Daily caloric intake was calculated by dietitian following the chart record.

### Outcome Measures

The primary outcome measure was the occurrence of febrile neutropenia (FN) and documented infection including bacteremia, pneumonia and central venous catheter infection in the neutropenic period. Secondary outcome measurements were organ dysfunction in the neutropenic period, acute GVHD, overall survival (OS) and nonrelapse mortality (NRM). Organ dysfunction was defined with reference to van den Berghe (*5–7*) as follows: 1) hypercreatininemia: serum creatinine level ≥2.0 mg/dL or more than twice the baseline; 2) hyperbilirubinemia: serum total bilirubin level ≥2.0 mg/dL; and 3) increased inflammatory markers: serum C-reactive protein (CRP) level ≥15 mg/dL. Acute GVHD was graded by the Consensus Criteria (*15*).

### Statistical Analyses

Standard descriptive statistics were used. The Student's *t*-test, chi-square, and Wilcoxon rank-sum tests were used to compare clinical and patient characteristics. Multiple logistic regression analysis was conducted to ascertain odds ratios (ORs) and 95% confidence intervals (CIs). OS was estimated using Kaplan-Meier curves. The cumulative incidences of NRM were estimated based on a Cox regression model for the cause-specific hazards by treating progressive disease or relapse as a competing event. Cox proportional hazard models were used for multivariate analysis of variables on NRM and OS after HCT. Clinical factors that were assessed for their association with NRM and OS included patient age, sex, conditioning regimen (TBI-based vs. non-TBI-based), donor [human leukocyte antigen (HLA)-matched vs. HLA-mismatched, related vs. unrelated], GVHD prophylaxis (cyclosporine-based vs. tacrolimus-based) and disease risk (standard vs. high). Factors with $P<0.10$ in the univariate analyses were subjected to a multivariate analysis. A level of $P<0.05$ was defined as statistically significant. All $P$ values are two-sided. All analyses were performed using SPSS 10.0 statistical software (Chicago, IL).

## RESULTS

### Patients and Transplantation Characteristics

The median ages of the patients in the normoglycemia, mild hyperglycemia, and moderate/severe hyperglycemia groups were, respectively, 31, 36, and 45 years. The percentages of patients who received graft from an unrelated donor were 32%, 51%, and 79%, and the percentages of patients who received GVHD prophylaxis with tacrolimus were 14%, 33%, and 74%. To clarify the risk factor to be included in moderate and severe hyperglycemia group, logistic analysis was performed, which showed older age and GVHD prophylaxis with tacrolimus were associated with moderate and severe hyperglycemia [$P=0.04$, OR 3.9 (1.1–14.0), and $P=0.01$, OR 5.5 (1.5–20.3), respectively], and there was a trend that patients who received stem cell from unrelated donor were associated with moderate and severe hyperglycemia [$P=0.07$, OR 3.6 (0.9–14.2)]. Multiple logistic analysis showed age more than 40 years old and GVHD prophylaxis with tacrolimus were associated with moderate and severe hyperglycemia [$P=0.042$, OR 4.1 (1.1–15.7), and $P=0.01$, OR 5.8 (1.5–22.1), respectively].

Although in practice we generally keep the parenteral glucose dose relatively low to avoid severe metabolic complications including hyperglycemia and hyperlipidemia during the acute phase of allogeneic HSCT, the possibility that the dose of parenteral nutrition affects the blood glucose level should be explored. We calculated the total caloric intake by combining both oral and parenteral nutrition. Although the mild hyperglycemia group received significantly more parenteral nutrition than the normoglycemia group (group 1 694+322 kcal/day vs. group 2 969+383 kcal/day), overall there was no essential difference in caloric intake between the three groups (1070+303 kcal/day, 1190+393 kcal/day, 1045+530 kcal/day, respectively). The median duration of the follow-up time in surviving patients was 809 days (range, 132-1530 days) in group 1, 369 days (105–1550 days) in group 2, and 587 days (170–774 days) in group 3. Described as hydrocortisone-equivalent dose, the median dose of corticosteroid used during neutropenia was 0 mg (0–1610 mg) in group 1, 100 mg (0–9700 mg) in group 2, and 375 mg (0–2468 mg) in group 3. Statistically more dose of corticosteroid was used in group 2 and group 3, compared with group 1.

### Primary Endpoints

The incidence of FN and documented infections is summarized in Table 2. The incidences of FN and documented infections including bacteremia, pneumonia, and central venous catheter infection in groups 1, 2 and 3 were, respectively, 89% and 32% (25%, 4% and 11%), 88% and 20% (16%, 6% and 6%), and 98% and 43% (36%, 14% and 14%). Overall, no statistically significant difference was observed between the three groups in the incidence of infectious episodes, including FN and documented infections.

**TABLE 2.** Endpoints

| Variable | Normoglycemia (<110 mg/dl) | Mild hyperglycemia (110–150 mg/dl) | Moderate and severe hyperglycemia (>150 mg/dl) |
|---|---|---|---|
| N | 28 | 49 | 14 |
| Febrile neutropenia | 23 (89) | 43 (88) | 13 (98) |
| Documented infection | 9 (32) | 10 (20) | 6 (43) |
| Bacteremia | 7 (25) | 8 (16) | 5 (36) |
| Pneumonia | 1 (4) | 3 (6) | 2 (14) |
| Central-venous catheter infection | 3 (11) | 3 (6) | 2 (14) |
| Organ dysfunction | | | |
| Hypercreatininemia | 1 (4) | 4 (8) | 4 (29) |
| Hyperbilirubinemia | 3 (11) | 11 (22) | 6 (43) |
| Increased inflammatory markers | 4 (14) | 15 (31) | 9 (64) |

Data are n (%).
Hypercreatininemia, serum creatinine level ≥2.0 mg/dl or more than twice of baseline; hyperbilirubinemia, serum bilirubin level ≥2.0 mg/dl; increased inflammatory markers, serum C-reactive protein level ≥15 mg/dl.

## Secondary Endpoints

The incidence of hypercreatininemia was 4% in group 1, 8% in group 2 and 29% in group 3, as summarized in Table 2, and that in group 3 was significantly higher than those in group 1 (OR 10.8, 95% CI 1.1–108.6; $P=0.018$) and group 2 (OR 4.5, 95% CI 1.0–21.1; $P=0.043$). The incidence of hyperbilirubinemia was, respectively, 11%, 22% and 43%, in the three groups, and that in group 3 was significantly higher than that in group 1 (OR 6.3, 95% CI 1.3–30.9; $P=0.017$). The incidence of increased inflammatory markers was, respectively, 14%, 31% and 64%, and that in group 3 was significantly higher than those in group 1 (OR 10.8, 95% CI 2.4–49.5; $P<0.001$) and group 2 (OR 4.1, 95% CI 1.2–14.3; $P=0.022$). Multiple logistic regression analysis showed that the degree of hyperglycemia was associated with hypercreatininemia, hyperbilirubinemia, and increased inflammatory markers (Table 3).

**TABLE 3.** Multiple logistic regression analysis for organ dysfunction and multiple variate analysis for acute GVHD, nonrelapse mortality, and overall survival

| Outcomes and variables | Odds/hazard ratio | 95% CI | *P* value |
|---|---|---|---|
| Multiple logistic regression analysis | | | |
| Hypercreatininemia | | | |
| Hyperglycemia | 5.2 | 1.1–24.6 | 0.039 |
| Hyperbilirubinemia | | | |
| Hyperglycemia | 4.9 | 1.6–14.9 | 0.005 |
| Increased inflammatory markers | | | |
| Hyperglycemia | 6.7 | 2.2–20.3 | 0.001 |
| Tacrolimus-based | 6.9 | 1.6–30.5 | 0.011 |
| Multivariate analysis (Cox-proportional hazard model) | | | |
| Acute GVHD | | | |
| Hyperglycemia | 2.3 | 1.2–4.3 | 0.013 |
| Disease risk (high) | 2.3 | 1.0–5.1 | 0.047 |
| HLA mismatch | 2.8 | 1.3–5.9 | 0.009 |
| Nonrelapse mortality | | | |
| Hyperglycemia | 2.9 | 1.2–6.6 | 0.013 |
| Disease risk (high) | 2.7 | 0.9–8.7 | 0.091 |
| Overall survival | | | |
| Hyperglycemia | 2.0 | 1.1–3.6 | 0.019 |
| TBI-containing | 2.3 | 1.1–5.0 | 0.035 |
| Disease risk (high) | 1.9 | 0.9–4.1 | 0.10 |

Odds ratios are presented for multiple logistic regression analysis; hazard ratios are presented for multivariate analysis.
GVHD, graft versus host disease; TBI, total body irradiation.

The cumulative incidence of grade II–IV acute GVHD is shown in Figure 1. The degree of hyperglycemia was associated with a higher incidence of grade II–IV acute GVHD

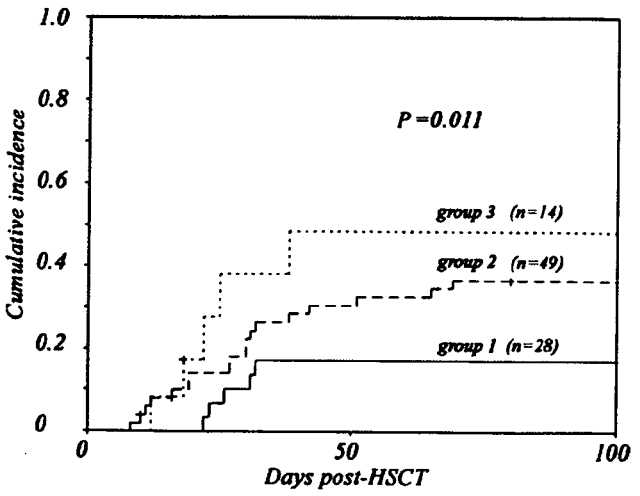


**FIGURE 1.** Cumulative incidence of acute GVHD grade II–IV stratified according to the mean glucose level during neutropenia. Group 1 included patients with normoglycemia, group 2 included patients with mild hyperglycemia, and group 3 included patients with moderate and severe hyperglycemia.

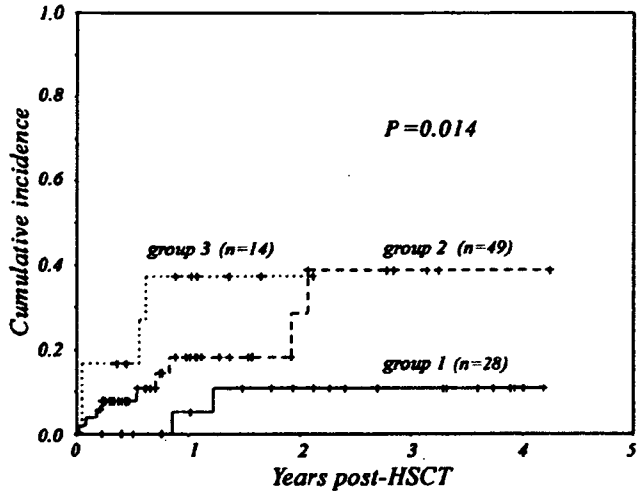


**FIGURE 2.** Cumulative incidence of treatment-related mortality stratified according to the mean glucose level during neutropenia.

($P$=0.002). A Cox proportional hazard model showed that hyperglycemia, high-risk underlying disease, and HLA mismatch were risk factors for grade II-IV acute GVHD (Table 3).

The cumulative incidence of NRM was, respectively, 5%, 17%, and 35% at 1 year, and was significantly related to the degree of hyperglycemia ($P$=0.014; Fig. 2). The probability of OS was, respectively, 88%, 70%, and 56%, and was significantly associated with hyperglycemia ($P$=0.008; Fig. 3). A Cox proportional hazard model showed that the degree of hyperglycemia was associated with NRM and OS (Table 3).

## DISCUSSION

In this study, we evaluated whether hyperglycemia during the cytopenic period after conditioning for HSCT could be a significant risk factor for the subsequent clinical course. Infectious diseases remain a major cause of morbidity and mortality in patients who receive HSCT, and we speculated that this might be exaggerated in the presence of hyperglycemia. Alternatively, hyperglycemia can be caused by infectious diseases and also aggravates infectious diseases to lead to a vicious cycle, with resultant morbidities that include organ dysfunction and mortality. Theoretically, strict glucose control should prevent this vicious cycle and help to reduce morbidity and mortality in patients after HSCT, as shown previously in ICU settings (*1, 2*). However, in this study the incidences of FN and documented infections were not different among the three groups. On the other hand, we found that hyperglycemia was associated with organ dysfunction and increased inflammatory markers, which was consistent with previous reports that demonstrated the impact of hyperglycemia on clinical outcomes of patients suffering from nonhematological diseases (*1–3, 12–14*). Additionally, a multivariate analysis showed that hyperglycemia was a risk factor for acute GVHD.

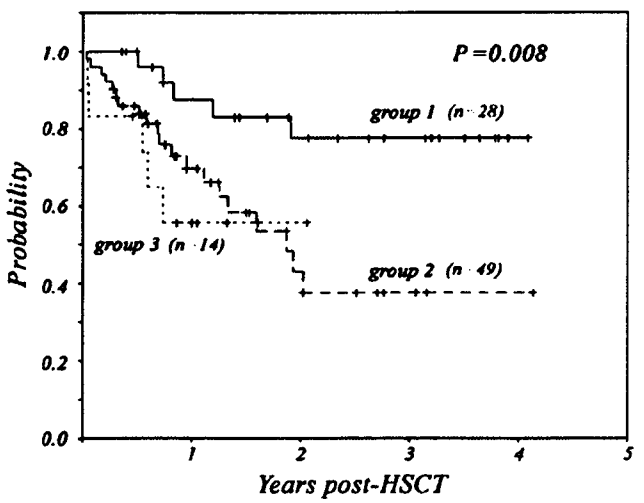


**FIGURE 3.** Overall survival stratified according to the mean glucose level during neutropenia.

The reason for the association between early hyperglycemia and late complications needs to be clarified. The increase in the levels of circulating cytokines due to hyperglycemia may further aggravate hyperglycemia itself (*16–21*). Therefore, this condition which occurs during the critical period of neutropenia before engraftment may influence the afferent phase of acute GVHD, as suggested by Ferrara et al. Elevated cytokine levels during the afferent phase then lead to subsequent acute GVHD in the effector phase (*22, 23*). Teshima et al. reported that the effector phase of acute GVHD is not antigen-specific and inflammatory cytokines mediate target destruction (*24*), and other reports have shown that inflammatory cytokines were required in acute GVHD and these molecules can cause tissue damage (*25–27*). With these reports in mind, it is reasonable to speculate that the aggravated production of inflammatory cytokines by hyperglycemia may be a risk factor in the pathogenesis of acute GVHD and organ dysfunction.

This study has several limitations, including heterogeneous patient populations and a retrospective nature. First, hyperglycemia can be caused by infection itself and it has been previously shown that the level of hyperglycemia was correlated with the severity of illness (*4*). In this retrospective study, we could not confirm whether hyperglycemia directly influenced organ dysfunction or increased inflammatory markers. Furthermore, statistically more corticosteroid was used in the group of moderate and severe hyperglycemia, and statistically more parenteral nutrition was used in the group of mild hyperglycemia. However, the observation that hyperglycemia and the severity of illness were independently associated with a worse prognosis has been well confirmed in the ICU setting (*4*), and several prospective studies have shown that intensive glucose control reduced both morbidity and mortality (*1, 2*). Considering these findings, we suggest that our data still support the possibility that the degree of hyperglycemia was associated with morbidity and mortality in the allogeneic HSCT setting. Second, we must consider that the patients who developed moderate and severe hyperglycemia included older patients, those who received more unrelated grafts, and those who received tacrolimus compared to other groups. In terms of immunosuppressive drugs, tacrolimus has recently become a preferred immunosuppressive drug for GVHD prophylaxis in unrelated or HLA-mismatched HSCT, based on the results of two Japanese studies, which showed that, compared to cyclosporine, tacrolimus was associated with a lower incidence of acute GVHD and better overall survival, which were similar to those in related HSCT, even

after HSCT with alternative donors, including unrelated donors (*28, 29*). Therefore, the effect of unrelated graft and tacrolimus on the incidence of acute GVHD and NRM might not be significant in this study.

The effects of tacrolimus on hyperglycemia, hyperbilirubinemia, and hypercreatininemia need to be clarified. It is well known that hyperglycemia occurs more often in patients receiving tacrolimus than in those receiving cyclosporine (*30–32*). In the present study, patients receiving tacrolimus were more likely to have moderate to severe hyperglycemia. However, the association of hyperbilirubinemia with tacrolimus has not been previously reported and two other studies (*33, 34*) showed that cyclosporine was more likely to cause hyperbilirubinemia than tacrolimus after allogeneic HSCT or kidney transplantation. Although the relative nephrotoxicity attributed to tacrolimus compared to cyclosporine has been controversial (*30, 33, 35*), studies that have reported such nephrotoxicity used a higher target tacrolimus level (>20 ng/ml) (*30, 35*). On the other hand, it has been reported that the use of lower levels of tacrolimus (10–15 ng/ml in our hospital) was associated with reduced complications in allogeneic HSCT (*36, 37*), with no difference in the incidence of hypercreatininemia compared to cyclosporine (*33*). Based on a consideration of all of these results, we think that tacrolimus might not be the direct cause of hypercreatininemia in this study. Finally, due to the nature of this retrospective study, during the period evaluated we did not apply any consistent protocol for glucose control and nutritional support, although we tried to avoid severe hyperglycemia (BG ≥200 mg/dl), which certainly biases the interpretation of the data, although it has been reported that the overall glucose level, rather than the dose of insulin administered, directly influenced the outcome of patients (*3*).

Even with these limitations, we believe that our observation is still of value in considering the clinical impact of the strict control of hyperglycemia during the early phase of HSCT. To confirm our preliminary observation, a prospective pilot study is underway to assess the effect of intensive glucose control after HSCT. If this pilot study shows a beneficial effect of intensive glucose control, a prospective randomized trial would be warranted to confirm the possibility that intensive glucose control improves the outcome after HSCT. Additionally, in this ongoing pilot study, we evaluate the diurnal blood glucose and insulin levels, including postprandial levels, to detect hyperglycemia more precisely before transplantation since the level of HgA1c is affected by both the blood glucose level and the turnover rate of red blood cells, and would not precisely correlate with the true mean blood glucose level in patients who received courses of blood transfusion for anemia.

In conclusion, the association of the degree of hyperglycemia during neutropenia and an increased risk of post-transplant complications and NRM was suggested, but the possibility that intensive glucose control improves the outcome after HSCT would only be confirmed in a prospective randomized trial.

## ACKNOWLEDGMENTS

*We thank the medical, nursing, data processing, laboratory, and clinical staffs at the National Cancer Center Hospital for their important contributions to this study through dedicated care of the patients. The authors are indebted to Y. Iisaka and M. Kurita for their assistance with data collection. We also thank S. Saito for helping to prepare the manuscript.*

## REFERENCES

1. Van den Berghe G, Wouters P, Weekwrs F, et al. Intensive insulin therapy in the critically ill patients. *N Engl J Med* 2001; 345: 1359.
2. Van den Berghe G, Wilmer A, Hermans G, et al. Intensive insulin therapy in the medical ICU. *N Engl J Med* 2006; 354: 449.
3. Van den Berghe G, Wouters PJ, Bouillon R, et al. Outcome benefit of intensive insulin therapy in the critically ill: Insulin dose versus glycemic control. *Crit Care Med* 2003; 31: 359.
4. Krinsley JS. Association between hyperglycemia and increased hospital mortality in a heterogeneous population of critically ill patients. *Mayo Clin Proc* 2003; 78: 1471.
5. Krinsley JS. Effect of an intensive glucose management protocol on the mortality of critically ill adult patients. *Mayo Clin Proc* 2004; 79: 992.
6. Vogelzang M, Nijboer JM, van der Horst IC, et al. Hyperglycemia has a stronger relation with outcome in trauma patients than in other critically ill patients. *J Trauma* 2006; 60: 873.
7. Ingels C, Debaveye Y, Milants I, et al. Strict blood glucose control with insulin during intensive care after cardiac surgery: Impact on 4-years survival, dependency on medical care, and quality-of-life. *Eur Heart J* 2006; 27: 2716.
8. Jeschke MG, Klein D, Bolder U, Einspanier R. Insulin attenuates the systemic inflammatory response in endotoxemic rats. *Endocrinology* 2004; 145: 4084.
9. Brix-Christensen V, Andersen SK, Andersen R, et al. Acute hyperinsulinemia restrains endotoxin-induced systemic inflammatory response: An experimental study in a porcine model. *Anesthesiology* 2004; 100: 861.
10. Sheean PM, Freels SA, Helton WS, Braunschweig CA. Adverse clinical consequences of hyperglycemia from total parenteral nutrition exposure during hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 2006; 12: 656.
11. Sheean PM, Braunschweig C, Rich E. The incidence of hyperglycemia in hematopoietic stem cell transplant recipients receiving total parenteral nutrition: A pilot study. *J Am Diet Assoc* 2004; 104: 1352.
12. Fietsam R Jr., Bassett J, Glover JL. Complications of coronary artery surgery in diabetic patients. *Am Surg* 1991; 57: 551.
13. Ortiz A, Ziyadeh FN, Neilson EG. Expression of apoptosis-regulatory genes in renal proximal tubular epithelial cells exposed to high ambient glucose and in diabetic kidney. *J Invest Med* 1997; 45: 50.
14. Vanhorebeek I, De Vos R, Mesotten D, et al. Protection of hepatocyte mitochondrial ultrastructure and function by strict blood glucose control with insulin in critically ill patients. *Lancet* 2005; 365: 53.
15. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant* 1995; 15: 825.
16. Cavallo MG, Pozzilli P, Bird C, et al. Cytokines in sera from insulin-dependent diabetic patients at diagnosis. *Clin Exp Immunol* 1991; 86: 256.
17. Morohoshi M, Fujisawa K, Uchimura I, Numano F. Glucose-dependent interleukin 6 and tumor necrosis factor production by human peripheral blood monocytes in vitro. *Diabetes* 1996; 45: 954.
18. Borst SE. The role of TNF-alpha in insulin resistance. *Endocrine* 2004; 23: 177.
19. Esposito K, Nappo F, Marfella R, et al. Inflammatory cytokine concentrations are acutely increased by hyperglycemia in humans: Role of oxidative stress. *Circulation* 2002; 106: 2067.
20. Hotamisligil GS, Murray DL, Choy LN, Spiegelman BM. Tumor necrosis factor α inhibits signaling from the insulin receptor. *Proc Natl Acad Sci USA* 1994; 91: 4854.
21. Tsigos C, Papanicolaou DA, Kyrou I, et al. Dose-dependent effects of recombinant human interleukin-6 on glucose regulation. *J Clin Endocrinol Metab* 1997; 82: 4167.
22. Ferrara JL, Reddy P. Pathophysiology of graft-versus-host disease. *Semin Hematol* 2006; 43: 3.
23. Ferrara JL, Cooke KR, Teshima T. The pathophysiology of acute graft-versus-host disease. *Int J Hematol* 2003; 78: 181.
24. Teshima T, Ordemann R, Reddy P, et al. Acute graft-versus-host disease does not require alloantigen expression on host epithelium. *Nat Med* 2002; 8: 575.
25. Laster SM, Wood JG, Gooding LR. Tumor necrosis factor can induce both apoptotic and necrotic forms of cell lysis. *J Immunol* 1988; 141: 2629.
26. Schmaltz C, Alpdogan O, Muriglan SJ, et al. Donor T cell-derived TNF

is required for graft-versus-host disease and graft-versus-tumor activity after bone marrow transplantation. *Blood* 2003; 101: 2440.
27. Krenger W, Ferrara JL. Dysregulation of cytokines during graft-versus-host disease. *J Hematother* 1996; 5: 3.
28. Hiraoka A, Ohashi Y, Okamoto S, et al. Phase III study comparing tacrolimus (FK506) with cyclosporine for graft-versus-host disease prophylaxis after allogeneic bone marrow transplantation. *Bone Marrow Transplant* 2001; 28: 181.
29. Yanada M, Emi N, Naoe T, et al. Tacrolimus instead of cyclosporine used for prophylaxis against graft-versus-host disease improves outcome after hematopoietic stem cell transplantation from unrelated donors, but not from HLA-identical sibling donors: A nationwide survey conducted in Japan. *Bone Marrow Transplant* 2004; 34: 331.
30. Ratanatharathorn V, Nash RA, Przepiorka D, et al. Phase III study comparing methotrexate and tacrolimus (prograf, FK506) with methotrexate and cyclosporine for graft-versus-host disease prophylaxis after HLA-identical sibling bone marrow transplantation. *Blood* 1998; 92: 2303.
31. Webster AC, Woodroffe RC, Taylor RS, et al. Tacrolimus versus ciclosporin as primary immunosuppression for kidney transplant recipients: Meta-analysis and meta-regression of randomised trial data. *BMJ* 2005; 331: 810.
32. McAlister VC, Haddad E, Renouf E, et al. Cyclosporin versus tacrolimus as primary immunosuppressant after liver transplantation: a meta-analysis. *Am J Transplant* 2006; 6: 1578.
33. Woo M, Przepiorka D, Ippoliti C, et al. Toxicities of tacrolimus and cyclosporin A after allogeneic blood stem cell transplantation. *Bone Marrow Transplant* 1997; 20: 1095.
34. Margreiter R; European Tacrolimus vs Ciclosporin Microemulsion Renal Transplantation Study Group. Efficacy and safety of tacrolimus compared with ciclosporin microemulsion in renal transplantation: a randomised multicentre study. *Lancet* 2002; 359: 741.
35. Nash RA, Antin JH, Karanes C, et al. Phase 3 study comparing methotrexate and tacrolimus with methotrexate and cyclosporine for prophylaxis of acute graft-versus-host disease after marrow transplantation from unrelated donors. *Blood* 2000; 96: 2062.
36. Wingard JR, Nash RA, Przepiorka D, et al. Relationship of tacrolimus (FK506) whole blood concentrations and efficacy and safety after HLA-identical sibling bone marrow transplantation. *Biol Blood Marrow Transplant* 1998; 4: 157.
37. Przepiorka D, Nash RA, Wingard JR, et al. Relationship of tacrolimus whole blood levels to efficacy and safety outcomes after unrelated donor marrow transplantation. *Biol Blood Marrow Transplant* 1999; 5: 94.

# Myeloablative allogeneic hematopoietic stem cell transplantation for non-Hodgkin lymphoma: a nationwide survey in Japan

Sung-Won Kim, Tetsuya E. Tanimoto, Noriyuki Hirabayashi, Seiichi Goto, Masahiro Kami, Satoshi Yoshioka, Toshiki Uchida, Kenji Kishi, Yuji Tanaka, Akio Kohno, Masaharu Kasai, Masakazu Higuchi, Masanobu Kasai, Shin-ichiro Mori, Takahiro Fukuda, Koji Izutsu, Hiroshi Sao, Takayuki Ishikawa, Tatsuo Ichinohe, Kengo Takeuchi, Kinuko Tajima, Ryuji Tanosaki, Mine Harada, Shuichi Taniguchi, Kensei Tobinai, Tomomitsu Hotta, and Yoichi Takaue

**We retrospectively surveyed the data of 233 patients who underwent myeloablative allogeneic hematopoietic stem cell transplantation (allo-HSCT) for non-Hodgkin lymphoma (NHL). Donors were HLA-matched relatives in 154 patients (66%) or unrelated volunteers in 60 (26%). Ninety patients (39%) were in complete remission. One hundred ninety-three (83%) received a total body irradiation (TBI)–based regimen, and 40 (17%) received a non–TBI-based regimen. Acute graft-versus-host disease (GVHD) occurred in 155 (67%) of the 233 evaluable patients; grade II to IV in 90 (39%), and grade III to IV in 37 (16%). Treatment-related mortality (TRM) was observed in 98 patients (42%), and 68% of them were related to GVHD. In a multivariate analysis, chemoresistance, prior autograft, and chronic GVHD were identified as adverse prognostic factors for TRM. Relapse or progression of lymphoma was observed in 21%. The 2-year overall survival rates of the patients with indolent (n = 38), aggressive (n = 111), and lymphoblastic lymphoma (n = 84) were 57%, 42%, and 41%, respectively. In a multivariate analysis, chemoresistance, prior autograft, and prior radiotherapy were identified as adverse prognostic factors for overall survival. Although myeloablative allo-HSCT represents an effective therapeutic option for patients with NHL, more work is still needed to decrease TRM and relapse. (Blood. 2006;108:382-389)**



## Introduction

Hematopoietic stem cell transplantation (HSCT) for patients with non-Hodgkin lymphoma (NHL) has been mainly focused on an autograft strategy. High-dose therapy with autologous HSCT (auto-HSCT) can increase remission rates and possibly prolong disease-free survival and overall survival (OS) in patients with chemotherapy-sensitive NHL at relapse.[1] This is also effective as first-line therapy for those with advanced aggressive lymphoma.[2] Nevertheless, relapse is a frequent cause of treatment failure after auto-HSCT.[1,3]

Allogeneic HSCT (allo-HSCT) has several advantages over auto-HSCT, because the former can avoid the reinfusion of malignant cells and can also be associated with a graft-versus-lymphoma (GVL) effect, which might reduce the risk of relapse. Most physicians believe that a small fraction of patients with end-stage aggressive lymphoma can still achieve prolonged lymphoma-free survival with the application of allo-HSCT. However, the high incidence of treatment-related mortality (TRM) (up to 55%) associated with allogeneic HSCT with a myeloablative regimen has prevented the wider application of this strategy.[4-8] Several reports on allo-HSCT for refractory or advanced lymphoma, as well as studies comparing auto- versus allo-HSCT for NHL, have been published over the past decade.[8-10] However, most of these studies were small and nonrandomized, and incorporated patients who had heterogeneous backgrounds. Thus, the role of allo-HSCT in the treatment of NHL remains controversial. Moreover, the outcome of allo-HSCT in each histologic subtype has not been fully determined. Previous studies have suggested that allo-HSCT improves the prognosis of patients with advanced follicular lymphoma (FL),[7,10,11] whereas few reports have been published on its benefit in aggressive lymphoma.[12,13] In particular, there has been very little information available on subtypes, including mantle-cell lymphoma[11,14]; peripheral T-cell lymphoma, unspecified (PTCL)[15]; natural killer (NK) cell lymphoma[16]; and anaplastic large cell lymphoma.

The application of reduced-intensity stem cell transplantation (RIST) or "nonmyeloablative" HSCT has been reported to decrease

From the Hematology and Hematopoietic Stem Cell Transplantation Division, National Cancer Center Hospital, Tokyo, Japan; the Department of Hematology, Tottori University Hospital, Tottori, Japan; the Department of Internal Medicine, Nagoya Daini Red Cross Hospital, Nagoya, Japan; the Department of Hematology/Oncology, Kyoto University Hospital, Kyoto, Japan; the Division of Hematology, Tokai University Hospital, Isehara, Japan; the Department of Hematology, Tokyo Metropolitan Komagome Hospital, Tokyo, Japan; the Department of Hematology and Oncology, JA Aichi Showa Hospital, Konan, Japan; the Department of Internal Medicine, Sapporo Hokuyu Hospital, Sapporo, Japan; the Department of Hematology, Hamanomachi Hospital, Fukuoka, Japan; the Department of Cell Therapy and Transplantation Medicine, University of Tokyo, Tokyo, Japan; the Department of Hematology, Meitetsu Hospital, Nagoya, Japan; the Department of Pathology, Cancer Institute of Japanese Foundation for Cancer Research, Tokyo, Japan; the First Department of Internal Medicine (Medicine and Biosystemic Science), Kyushu University Graduate School of Medical Sciences, Fukuoka, Japan; and the Department of Hematology, Toranomon Hospital, Tokyo, Japan.

Submitted March 1, 2005; accepted December 22, 2005. Prepublished online as *Blood* First Edition Paper, March 7, 2006; DOI 10.1182/blood-2005-02-0596.

Supported in part by grants from the Ministry of Health, Labor and Welfare, Japan.

Presented in part as a poster presentation at the 44th annual meeting of the American Society of Hematology, Philadelphia, PA, December 2002.

**Reprints:** Yoichi Takaue, Medical Oncology, National Cancer Center Hospital, 5-1-1, Tsukiji, Chuo-Ku, Tokyo 104-0045, Japan; e-mail: ytakaue@ncc.go.jp.

The publication costs of this article were defrayed in part by page charge payment. Therefore, and solely to indicate this fact, this article is hereby marked "advertisement" in accordance with 18 U.S.C. section 1734.

TRM.[17-19] Additionally, the recent development of supportive treatments may have decreased the risk of TRM and facilitated the application of allo-HSCT to NHL.[20] Therefore, we conducted a retrospective nationwide survey on Japanese patients with NHL who had undergone conventional allo-HSCT to establish a benchmark of myeloablative allo-HSCT in the treatment of NHL.

## Patients, materials, and methods

### Data sources

This survey collected the data of 233 consecutive patients who received myeloablative allo-HSCT for NHL between 1990 and 2001 in 56 participating hospitals. Data were derived from questionnaires distributed to each hospital. Additional questionnaires were sent to confirm the follow-up data, including the occurrence of graft-versus-host disease (GVHD). The indications for allo-HSCT were left to the discretion of each institution. The patients included in this study received a conditioning regimen with an intensity that was equivalent to that of total body irradiation (TBI) plus cyclophosphamide or busulfan plus cyclophosphamide. Patients who had previously received monoclonal antibody therapy or T-cell–depleted transplantation, those younger than 14 years, and those who received RIST were not included. Additionally, those with adult T-cell leukemia/lymphoma were excluded because their clinical course differed from that of other types of lymphoma. The minimum data required for the inclusion of a patient in this study were age, sex, histologic diagnosis, prior treatment details, status at transplantation, donor information, conditioning regimen, date of transplantation, therapy-related complications, date of last follow-up, disease status at follow-up, date of disease progression/death, and cause of death. Approval was obtained from the institutional review board. Informed consent was provided according to the Declaration of Helsinki.

### Definitions

The initial institutional histologic diagnosis was further reviewed by a pathologist (K. Takeuchi) using the WHO classification.[21] Briefly, NHL was divided into 3 clinical subtypes: indolent, aggressive, and lymphoblastic lymphoma. Indolent lymphoma included all grades of FL and extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue (MALT lymphoma). Aggressive lymphoma included all lymphomas except for indolent and lymphoblastic lymphoma. Transformed indolent lymphoma and Burkitt lymphoma were classified as aggressive lymphoma. Furthermore, because most of the patients were evaluated before publication of the WHO classification, this analysis only included those who had tumors that formed lesions, such as T-cell lymphoblastic lymphoma (T-LBL), and all other patients who had features of leukemia were excluded. Those with chemosensitive disease included all patients who had shown a response to the last chemotherapy prior to transplantation (partial remission [PR], complete remission [CR] unconfirmed, and CR), whereas chemoresistant disease included those with primary refractory disease or refractory relapse prior to transplantation. Acute and chronic GVHD was graded according to the consensus criteria.[22,23] Patients who survived 100 days were evaluable for the assessment of chronic GVHD. OS was measured as the time from the day of transplantation until death from any cause, and progression-free survival (PFS) was the time from the day of transplantation until disease progression (PD)/relapse or death from any cause. Patients who died from transplantation-related causes were classified as TRM regardless of their disease status.

### Statistical analysis

OS and PFS were calculated using the Kaplan-Meier method.[24] Surviving patients were censored on the last day of follow-up, in July 2002. The associations among patient-, disease-, and transplantation-related factors and OS were assessed by using univariate and multivariate Cox proportional hazards models. The associations between these factors and TRM were assessed by using univariate and multivariate logistic models. The variables analyzed included age, clinical subtype, histologic diagnosis, chemosensitivity, history of autograft or radiotherapy, years of transplantation, donor, source of stem cells, TBI-containing regimen, GVHD prophylaxis, and acute and chronic GVHD. Acute GVHD was treated as a time-dependent covariate in the Cox model. Stepwise variable selection at a significance level of .05 was used to identify covariates associated with outcomes. TRM and disease progression/relapse were calculated by using cumulative incidence. The statistical analysis was performed with the SAS 8.2 program package (SAS Institute, Cary, NC).

## Results

### Patients' characteristics

The patients' characteristics are listed in Table 1. All patients were younger than 60 years at the time of transplantation, with a median age of 31 years. Thirty-eight patients (16%) had indolent lymphoma, 111 (48%) had aggressive lymphoma (diffuse large B-cell, n = 44; PTCL, n = 22; extranodal NK/T-cell, n = 19; anaplastic large cell, n = 7; mantle cell, n = 5; Burkitt, n = 4; angioimmunoblastic T cell, n = 2; blastic NK cell, n = 2; hepatosplenic T-cell, n = 2; subcutaneous panniculitis like T cell, n = 2; mycosis fungoides with visceral dissemination, n = 2), and 84 (36%) had lymphoblastic lymphoma. Ninety patients (39%) were in CR, 38 (16%) were in PR, 42 (18%) were in primary refractory, and 63 (27%) had refractory relapse at the time of allo-HSCT. Ninety patients (39%) had received 4 or more chemotherapy regimens before allo-HSCT. Forty patients (17%) had received prior autograft, and 81 (35%) had received prior radiotherapy. One hundred fifty-four patients (66%) received a transplant from a human leukocyte antigen (HLA)–matched related donor, 19 (8%) from a 1-antigen–mismatched related donor, 43 (19%) from a matched unrelated donor, and 17 (7%) from a 1-antigen–mismatched unrelated donor. One hundred fifty-nine (68%) patients received bone marrow (60 from an unrelated donor) and 70 (30%) received granulocyte colony-stimulating factor (G-CSF)–mobilized peripheral blood. One hundred ninety-three patients (83%) received TBI-based myeloablative regimens, including TBI 12 Gy plus cyclophosphamide (n = 60); a combination of TBI, cyclophosphamide, and etoposide (n = 47); or TBI, cyclophosphamide, and cytarabine (n = 40). Forty patients (17%) received a non–TBI-based myeloablative regimen, including a combination of busulfan and cyclophosphamide with or without other agents (n = 27); melphalan, thiotepa, and busulfan (n = 3); cytarabine, ranimustine, carboplatin, cyclophosphamide, and total lymphoid irradiation (n = 2); or cytarabine, etoposide, and busulfan (n = 2). The remaining 6 patients received individualized regimens. GVHD prophylaxis included a combination of cyclosporin and methotrexate in 204 (88%) or tacrolimus and methotrexate in 22 (9%). Two hundred twenty-six patients (97%) were treated with G-CSF, starting at days +1 to +6 after graft infusion until engraftment.

### GVHD

Acute GVHD occurred in 155 (67%) of the 233 patients: grade I in 65 (28%), grade II to IV in 90 (39%), and grade III to IV in 37 (16%) patients. Of the 165 patients who survived the initial 100 days after allo-HSCT, chronic GVHD occurred in 79 (48%), with extensive type in 48 (29%). In allo-HSCT from related (n = 173) and unrelated (n = 60) donors, grade II to IV acute GVHD occurred, respectively, in 61 (35%) and 30 (50%), grade III to acute GVHD occurred in 25 (15%) and 12 (20%), chronic GVHD occurred in 54 (31%) and 25 (42%) patients, and chronic extensive

**Table 1. Patient-, disease-, and transplantation-related characteristics**

| Variable | No. (%)* |
|---|---|
| **Patient characteristics** | |
| Younger than 40 y | 158 (68) |
| 40 y or older | 75 (32) |
| Male sex | 150 (64) |
| **Disease characteristics at diagnosis** | |
| Histology | |
| Indolent | 38 (16) |
| Follicular | 37 (16) |
| MALT | 1 (0) |
| Aggressive | 111 (48) |
| Diffuse large B cell | 44 (19) |
| Peripheral T cell, unspecified | 22 (9) |
| Extranodal NK/T cell, nasal type | 19 (8) |
| Anaplastic large cell | 7 (3) |
| Mantle cell | 5 (2) |
| Others | 14 (6) |
| Lymphoblastic | 84 (36) |
| Precursor B cell | 7 (3) |
| Precursor T cell | 77 (33) |
| Stage I | 9 (4) |
| Stage II | 25 (11) |
| Stage III | 30 (13) |
| Stage IV | 150 (64) |
| No data | 19 (8) |
| **Disease characteristics at transplantation** | |
| Response to chemotherapy† | |
| Sensitive | 128 (55) |
| Complete remission‡ | 90 (39) |
| Partial remission | 38 (16) |
| Resistant | 104 (45) |
| Primary refractory disease | 41 (18) |
| Refractory relapse | 63 (27) |
| No. of prior chemotherapy regimens† | 3 (0-11) |
| Fewer than 4 regimens | 143 (61) |
| At least 4 regimens | 90 (39) |
| Prior autograft | 40 (17) |
| Prior radiotherapy | 81 (35) |
| **Transplantation characteristics** | |
| Year of transplantation | |
| 1990-1995 | 46 (20) |
| 1996-2001 | 187 (80) |
| No. of patients receiving a transplant per hospital | |
| Fewer than 9 patients | 146 (63) |
| At least 9 patients | 87 (37) |
| Donor | |
| HLA-matched related | 154 (66) |
| HLA-1 antigen-mismatched related | 19 (8) |
| HLA-matched unrelated | 43 (19) |
| HLA-1 antigen-mismatched unrelated | 17 (7) |
| Donor-recipient sex match | |
| Male-male | 80 (34) |
| Male-female | 66 (28) |
| Female-male | 33 (14) |
| Female-female | 46 (20) |
| Donor-recipient CMV status§ | |
| +/+ | 131 (57) |
| −/+ | 14 (6) |
| +/− | 14 (6) |
| −/− | 11 (5) |
| Source of stem cells | |
| Bone marrow | 159 (68) |
| Peripheral blood cells | 70 (30) |
| Bone marrow + peripheral blood cells | 2 (1) |
| Cord blood | 2 (1) |
| Conditioning regimen | |
| TBI-containing | 193 (83) |
| Non-TBI | 40 (17) |
| GVHD prophylaxis | |
| Cyclosporin + methotrexate | 204 (88) |
| Tacrolimus + methotrexate | 22 (9) |
| Others | 7 (3) |

The study included 233 patients. The median age was 31 years (range, 15-59 years). Age was a continuous variable.

MALT indicates extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue; NK, natural killer; HLA, human leukocyte antigen; CMV, cytomegalovirus; TBI, total body irradiation; GVHD, graft-versus-host disease.

*Categoric variable.

†One patient with mediastinal B-LBL did not receive prior chemotherapy for an unknown reason but did receive prior radiotherapy.

‡Includes 2 patients in complete remission, unconfirmed.

§Sixty-three pairs were not evaluated for CMV status.

GVHD occurred in 33 (19%) and 16 (27%). In allo-HSCT from HLA-matched (n = 197) and mismatched (n = 36) donors, grade II to IV acute GVHD occurred, respectively, in 76 (39%) and 15 (42%), grade III to IV acute GVHD occurred in 30 (15%) and 7 (19%), chronic GVHD occurred in 65 (33%) and 14 (39%), and chronic extensive GVHD occurred in 41 (21%) and 7 (19%). The distribution pattern of the incidences of acute and chronic GVHD by background factors was analyzed by using a chi-square test. Although none of the factors correlated with acute GVHD, the incidence of chronic GVHD was higher in patients who had GVHD prophylaxis with tacrolimus plus methotrexate than in those with cyclosporin plus methotrexate ($P = .015$, chi-square test; $P = 0.023$, Fisher exact test).

### Disease response

Of the 143 patients who had measurable disease at allo-HSCT, 89 (62%) achieved CR, 7 (5%) PR, 6 (4%) stable disease (SD), and 12 (8%) PD, whereas 29 (20%) were not evaluable because of early death. Of the 90 patients who were in CR at transplantation, 80 (89%) maintained CR, 4 (4%) showed PD, and 6 (7%) were not evaluable because of early death. Thirty-five patients died before the first response evaluation, with a median survival of 29 days (range, 0-72 days) after allo-HSCT. In the 27 patients with indolent lymphoma who had measurable disease at allo-HSCT, 22 (81%) achieved CR or PR. In the 72 patients with aggressive lymphoma who had measurable disease at allo-HSCT, 49 (68%) achieved CR or PR. In the 41 patients with lymphoblastic lymphoma who had measurable disease at allo-HSCT, 26 (63%) achieved CR.

### TRM, disease relapse, and progression

Ninety-eight patients (42%) died of TRM, and its cumulative incidence is shown in Figure 1. Of the 98 patients who died of therapy-related complications, 60 (61%) died within day 100 of transplantation and 38 (39%) died thereafter. The major causes of TRM included GVHD (n = 11), infection (n = 29), interstitial pneumonitis (n = 16), venoocclusive disease of the liver (n = 11), thrombotic microangiopathy (n = 8), heart failure (n = 7), hemorrhage (n = 4), renal failure (n = 3), and others (n = 9), as shown in Table 2. The causes of infection-related mortality (n = 29) were bacterial (n = 13), fungal (n = 11), or viral (n = 5). Seventeen (59%) of 29 patients died of infections within 100 days of allo-HSCT, 7 (24%) from 101 days to 1 year and 5 (17%) thereafter. Fourteen patients died of TRM before engraftment. Of the 98 patients who died of TRM, 67 (68%) had GVHD, and 11 of

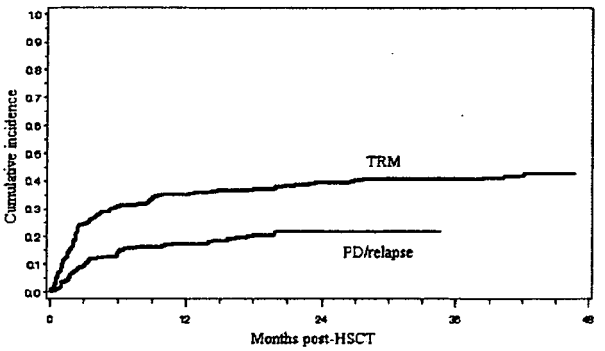


**Figure 1. Cumulative incidences of treatment-related mortality (TRM) and disease relapse/progression (PD/relapse).**

these died of GVHD (6 acute, 5 chronic) itself. The 14 factors shown in Table 3 were assessed with regard to their relation to TRM. A univariate analysis revealed that 6 factors, including older patient age, chemoresistant disease, prior autograft, prior radiotherapy, aggressive lymphoma other than PTCL, and chronic GVHD, were associated with a significantly increased risk of TRM. In a multivariate analysis using a logistic model, chemoresistant disease, prior autograft, and chronic GVHD remained significant.

The cumulative incidence of relapse and PD is shown in Figure 1. Relapse or progression of lymphoma after allo-HSCT was observed in 49 patients (21%; 5 indolent, 19 aggressive, 25 LBL), and 32 (14%; 3 indolent, 13 aggressive, and 16 LBL) died of PD. Of the 105 patients with chemoresistant disease before allo-HSCT, 61 (58%) died of treatment-related complications, 19 (18%) died of PD, and 25 (24%) are alive with a median follow-up of 20.9 months (range, 1.8-136.0 months). Of the 128 patients with chemosensitive disease before allo-HSCT, 37 (29%) died of treatment-related complications, 12 (9%) died of PD, and 79 (62%) are alive with a median follow-up of 35.2 months (range, 4.4-140.2 months). Eight (16%) of the 49 patients who showed PD died of treatment-related complications such as infection (n = 4), interstitial pneumonitis (n = 3), and GVHD (n = 1). Only 6 of the 70 patients who had passed 2 years after transplantation developed relapse thereafter.

### Donor lymphocyte infusion

Donor lymphocyte infusions (DLIs) were given after the withdrawal of immunosuppressive therapy to those who relapsed or showed evidence of disease progression or persistent disease without any sign of GVHD. A total of 7 patients, including 5 with T-LBL, received DLI after allo-HSCT from an HLA-matched related donor (n = 6) or a -matched unrelated donor (n = 1). Two patients who received DLI from an HLA-matched related donor developed grade II acute GVHD, which subsequently extended to extensive chronic GVHD; one of them with T-LBL died without a response, whereas the other with T-cell lymphoma is still alive without disease progression 3.8 years after allo-HSCT. Five patients did not develop GVHD following DLI; 3 patients subsequently died of disease progression, but 2 patients with T-LBL are still alive without disease progression at 361 and 783 days after allo-HSCT.

### OS and PFS

One hundred four (45%) of the 233 patients are currently alive with a median follow-up of 31 months (range, 1.8-138 months). The OS and PFS are, respectively, 45% and 40% at 2 years, and 39% and 36% at 5 years after allo-HSCT (Figure 2). Median OS and PFS are, respectively, 15.6 months (95% confidence interval, 9.6-27.6 months) and 9.6 months (6-18 months). The 2-year OS of those with indolent, aggressive, and lymphoblastic lymphoma was, respectively, 57%, 42%, and 41%. Patients with indolent lymphoma tended to have a better survival ($P$ = .131, log rank test; $P$ = .064, G. Wilcoxon test) (Figure 3). Kaplan-Meier estimates of OS of patients with 4 histologic subtypes of aggressive lymphoma, including diffuse large B-cell lymphoma (n = 44), PTCL (n = 22), extranodal NK/T-cell lymphoma, nasal type (n = 19), and others (n = 26), are shown in Figure 4.

The 14 clinical factors shown in Table 4 were assessed with regard to their relation to OS. A univariate analysis revealed that 5 factors, including chemoresistant disease, prior autograft, prior radiotherapy, aggressive lymphoma other than PTCL, and clinical subtype (aggressive versus indolent), were associated with a significantly worse OS. In a multivariate analysis using Cox proportional hazard models, chemoresistant disease, prior autograft, and prior radiotherapy were associated with a worse OS (Table 4). Acute GVHD, which was treated as a time-dependent variable, was not a significant factor for OS in both univariate and multivariate models. The relation between OS and response to chemotherapy is shown in Figure 5.

## Discussion

This report describes the general outcome of patients with NHL who underwent modern allo-HSCT with a myeloablative regimen

**Table 2. Causes of treatment-related mortality**

| Causes of TRM | Patients, no. (%) | No. of patients with GVHD | No. of patients without GVHD | Early death, no.* |
|---|---|---|---|---|
| GVHD | 11 (11) | | | |
| Infection | 29 (30) | 15 | 8 | 6 |
| Interstitial pneumonitis | 16 (17) | 15 | 0 | 1 |
| Venoocclusive disease | 11 (11) | 5 | 4 | 2 |
| Thrombotic microangiopathy | 8 (8) | 7 | 1 | 0 |
| Heart failure | 7 (7) | 3 | 1 | 3 |
| Hemorrhage | 4 (4) | 3 | 1 | 0 |
| Renal failure | 3 (3) | 2 | 1 | 0 |
| Others† | 9 (9) | 6 | 1 | 2 |
| Total | 98 (100) | 56 | 17 | 14 |

GVHD indicates graft-versus-host disease.

*Early death was defined as treatment-related death before engraftment.

†Others (n = 9) were acute respiratory distress syndrome (n = 2), hepatic failure (n = 2), leukoencephalopathy (n = 1), secondary solid cancer (n = 1), suicide (n = 1), and unknown cause (n = 2).

**Table 3. Univariate and multivariate analyses of treatment-related mortality**

| Variable | No. | Univariate analysis | | Multivariate analysis | |
|---|---|---|---|---|---|
| | | Hazard ratio (95% CI) | P | Hazard ratio (95% CI) | P |
| **Age at transplantation** | | | .035 | | — |
| Younger than 40 y | 158 | 1.00 | | — | |
| 40 y or older | 75 | 1.82 (1.04-3.17) | | — | |
| **Clinical subtype** | | | .349 | | — |
| Indolent | 38 | 1.00 | | — | |
| Lymphoblastic | 84 | 1.47 (0.66-3.32) | | — | |
| **Clinical subtype** | | | .103 | | — |
| Indolent | 38 | 1.00 | | — | |
| Aggressive | 111 | 1.91 (0.88-4.16) | | — | |
| **Aggressive lymphoma** | | | .045 | | — |
| PTCL | 22 | 1.00 | | — | |
| Non-PTCL | 89 | 2.85 (1.02-7.94) | | — | |
| **Response to chemotherapy** | | | < .001 | | < .001 |
| Sensitive | 128 | 1.00 | | 1.00 | |
| Resistant | 105 | 3.41 (1.97-5.88) | | 2.95 (1.66-5.25) | |
| **Prior autograft** | | | < .001 | | < .001 |
| No | 193 | 1.00 | | 1.00 | |
| Yes | 40 | 4.74 (2.23-10.07) | | 4.09 (1.85-9.04) | |
| **Prior radiotherapy** | | | .010 | | — |
| No | 152 | 1.00 | | — | |
| Yes | 81 | 2.05 (1.18-3.55) | | — | |
| **Years of transplantation** | | | .225 | | — |
| 1996-2001 | 187 | 1.00 | | — | |
| 1990-1995 | 46 | 1.49 (0.78-2.86) | | — | |
| **Donor** | | | .295 | | — |
| HLA-matched | 197 | 1.00 | | — | |
| HLA-mismatched | 36 | 1.46 (0.72-2.98) | | — | |
| **HLA-matched donor** | | | .437 | | — |
| Related | 154 | 1.00 | | — | |
| Unrelated | 43 | 1.24 (0.72-2.15) | | — | |
| **Source of stem cells*** | | | .544 | | — |
| BM | 159 | 1.00 | | — | |
| PBSCs | 70 | 1.09 (0.82-1.46) | | — | |
| **Conditioning regimen** | | | .144 | | — |
| TBI-containing | 193 | 1.00 | | — | |
| Others | 40 | 1.67 (0.84-3.30) | | — | |
| **GVHD prophylaxis†** | | | .169 | | — |
| Cyclosporin + methotrexate | 204 | 1.00 | | — | |
| Tacrolimus + methotrexate | 22 | 1.86 (0.77-4.51) | | — | |
| **Acute GVHD** | | | .537 | | — |
| No | 78 | 1.00 | | — | |
| Yes | 155 | 1.19 (0.69-2.06) | | — | |
| **Chronic GVHD** | | | < .001 | | .029 |
| No | 79 | 1.00 | | 1.00 | |
| Yes | 154 | 2.76 (1.53-4.98) | | 2.02 (1.07-3.77) | |

CI indicates confidence interval; PTCL, peripheral T-cell lymphoma; HLA, human leukocyte antigen; BM, bone marrow; GVHD, graft-versus-host disease; and —, not applicable.

*Those who received cord blood (n = 2) or BM + PBSC (n = 2) were excluded because of the small number of patients.

†Seven patients using other GVHD prophylaxis were excluded.

in Japan, focusing on the background problems of myeloablative therapy and the identification of risk factors for TRM and OS. We showed that long-term, lymphoma-free survival could be achieved in approximately 40% of patients. Patients with FL had a better prognosis, consistent with previous reports.[8,10] Even in patients with aggressive lymphoma or LBL, long-term survival of 35% was identified, consistent with previous reports.[8,9] However, there were no significant differences between clinical subtypes (eg, aggressive versus indolent or PTCL versus non-PTCL) in a multivariate analysis. Because rituximab became commercially available after 2001 in Japan, patients with B-cell NHL who received anti-CD20 antibody therapy were not included in this study. The clinical effect of the introduction of rituximab on outcome after allogeneic transplantation should be carefully evaluated in a future study.

Our study confirmed a high TRM rate (42%) after conventional allo-HSCT with a myeloablative regimen, consistent with previous reports.[4-8,25] One of the major causes of death was severe regimen-related toxicities, which included interstitial pneumonitis, venoocclusive disease, cardiac and renal toxicity, and organ hemorrhage. Although TBI-based regimens are frequently chosen because lymphoma cells are considered to be sensitive to irradiation, they have also been associated with long-term complications, including

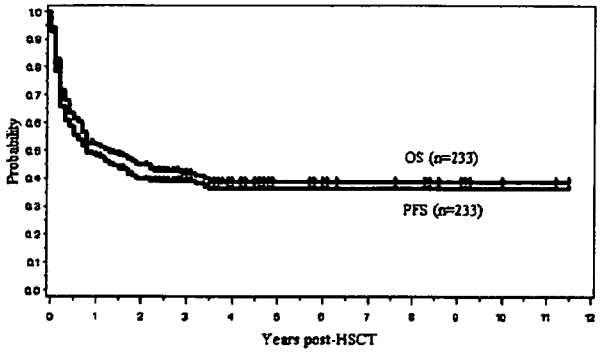


**Figure 2. Overall survival (OS) and progression-free survival (PFS) for all 233 patients.**

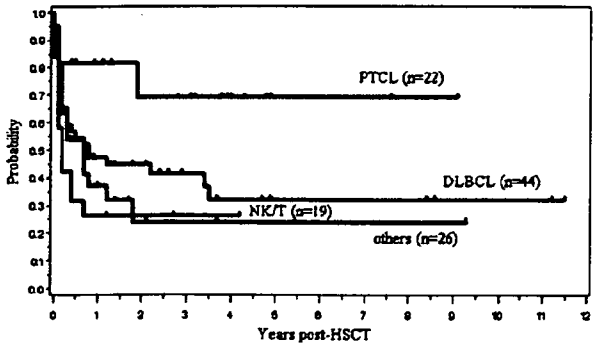


**Figure 4. Overall survival for patients with 4 histologic subtypes of aggressive lymphoma.** PTCL indicates peripheral T-cell lymphoma, unspecified; DLBCL, diffuse large B-cell lymphoma; NK/T, extranodal NK/T-cell lymphoma, nasal type.

interstitial pneumonitis.[26,27] Because most patients received TBI-based regimens as reported,[4,5,7] we failed to detect any significant differences in TRM between those who received or did not receive TBI.

Another major cause of death in our study was GVHD and/or infection. Of the 98 patients who died of treatment-related complications in our study, 29 (30%) died of infection. At least half of the patients (15 of 29) who died of infectious complications also had GVHD. In a prospective trial of allo-HSCT for patients with NHL, infection accounted for 63% of all TRM,[28] whereas other studies, including ours, have reported an incidence of 25% to 30%.[4,6] In practical transplantation procedures, complications are usually multifactorial, and it is always very difficult to define the exact cause of death, which may account for the wide variations in the incidence of infections among those who died of TRM (18%-63%) in previous reports.[4,5,28,29]

In this study, the incidence of chronic GVHD was high (48%), and chronic GVHD was a risk factor for TRM. The reason for the higher incidence of chronic GVHD in our study compared with the IBMTR report[9,30] was that the IBMTR study included data of patients who died within 100 days after allo-HSCT, whereas we excluded these patients. Unexpectedly, the incidence of chronic GVHD was higher in patients who had GVHD prophylaxis with tacrolimus plus methotrexate than in those with cyclosporin plus methotrexate. In Japan, there is a clear tendency to select tacrolimus rather than cyclosporine for GVHD prophylaxis in unrelated or HLA-mismatched transplantation.[31,32] In addition, PBSCT is not yet permitted for unrelated transplantation. Altogether, the higher incidence of GVHD observed in the tacrolimus group may simply reflect that patients with a higher risk of GVHD were selected to receive tacrolimus.

We found that the incidence of disease relapse/progression of NHL was low (21%). High TRM in the early phase of the transplantation course may mask later disease relapse/progression, and this made it difficult to estimate the relapse rate in this study. OS and PFS were not affected by the severity of acute GVHD. Our limited analysis failed to confirm a GVL effect after myeloablative allo-HSCT. Although the risk of relapse for patients with acute or chronic GVHD was not significantly different from that of patients without acute or chronic GVHD in previous studies with malignant lymphoma,[8,10,30] a study from the Japan Marrow Donor Program showed that the development of grade II to IV acute GVHD was associated with a lower incidence of disease progression after unrelated HSCT.[31] It has been reported that a low level of acute GVHD was associated with improved OS, and all levels of acute GVHD were associated with a decrease in the relapse rate for intermediate-grade NHL.[8] High levels of acute GVHD had a deleterious effect on OS but were associated with an improved relapse rate for LBL.[8] Thus, our study confirmed that greater effort is required to reduce GVHD-related complications after myeloablative allo-HSCT.

We confirmed that chemoresistance before allo-HSCT and prior autograft were significant risk factors for both OS and TRM. RIST or a less organ-toxic myeloablative allo-HSCT using a combination of fludarabine plus intravenous busulfan may be applied more safely in this population to reduce TRM.[19-21,33,34] However, further studies are needed to determine whether reduced-intensity conditioning could control activity of chemoresistant disease. In contrast to previous studies, we showed that prior radiotherapy was associated with a significantly worse OS, which may be related to the fact that 44 (54%) of the 81 patients who had a history of local radiotherapy had refractory disease at transplantation. Hence, it might be that prior radiotherapy was a marker of survival for more advanced and refractory disease.

In conclusion, we confirmed that myeloablative allo-HSCT is a curative therapeutic option in a subset of patients with NHL, but it carries a high risk of toxicities and TRM. Chemoresistant disease and a history of previous autograft are risk factors for both OS and TRM. Whether the introduction of a reduced-intensity transplantation procedure results in reduction of TRM should be evaluated, and more effective GVHD prophylaxis while maintaining a GVL effect should be developed.

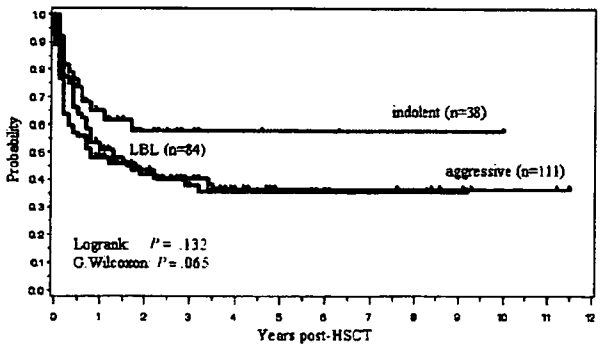


**Figure 3. Overall survival stratified according to the clinical subtype.** Indolent lymphoma included all grades of FL and extranodal marginal zone B-cell lymphoma of mucosa-associated lymphoid tissue. Aggressive lymphoma included all lymphomas except for indolent and lymphoblastic lymphoma (LBL).

**Table 4. Univariate and multivariate analyses of overall survival**

| Variable | No. | Univariate analysis Hazard ratio (95% CI) | Univariate analysis P | Multivariate analysis Hazard ratio (95% CI) | Multivariate analysis P |
|---|---|---|---|---|---|
| **Age at transplant** | | | .134 | — | — |
| Younger than 40 y | 158 | 1.00 | | — | — |
| 40 y or older | 75 | 1.32 (0.92-1.90) | | — | — |
| **Clinical subtype** | | | .126 | — | — |
| Indolent | 38 | 1.00 | | — | — |
| Lymphoblastic | 84 | 1.57 (0.88-2.80) | | — | — |
| **Clinical subtype** | | | .045 | — | — |
| Indolent | 38 | 1.00 | | — | — |
| Aggressive | 111 | 1.77 (1.01-3.11) | | — | — |
| **Aggressive lymphoma** | | | .004 | — | — |
| PTCL | 22 | 1.00 | | — | — |
| Non-PTCL | 89 | 3.45 (1.47-7.69) | | — | — |
| **Response to chemotherapy** | | | < .001 | — | — |
| Sensitive | 128 | 1.00 | | — | — |
| Resistant | 105 | 3.31 (2.30-4.76) | | 3.12 (2.16-4.51) | < .001 |
| **Prior autograft** | | | < .001 | — | — |
| No | 193 | 1.00 | | — | — |
| Yes | 40 | 2.59 (1.73-3.87) | | 2.18 (1.43-3.30) | < .001 |
| **Prior radiotherapy** | | | < .001 | — | — |
| No | 152 | 1.00 | | — | — |
| Yes | 81 | 1.99 (1.41-2.83) | | 1.47 (1.02-2.11) | .037 |
| **Years of transplantation** | | | .932 | — | — |
| 1996-2001 | 187 | 1.00 | | — | — |
| 1990-1995 | 46 | 1.02 (0.67-1.54) | | — | — |
| **Donor** | | | .076 | — | — |
| HLA-matched | 197 | 1.00 | | — | — |
| HLA-mismatched | 36 | 1.50 (0.96-2.33) | | — | — |
| **HLA-matched donor** | | | .769 | — | — |
| Related | 154 | 1.00 | | — | — |
| Unrelated | 43 | 0.93 (0.58-1.50) | | — | — |
| **Source of stem cells*** | | | .095 | — | — |
| BM | 159 | 1.00 | | — | — |
| PBSCs | 70 | 1.37 (0.95-2.00) | | — | — |
| **Conditioning regimen** | | | .107 | — | — |
| TBI-containing | 193 | 1.00 | | — | — |
| Others | 40 | 1.42 (0.93-2.17) | | — | — |
| **GVHD prophylaxis†** | | | .227 | — | — |
| Cyclosporin + methotrexate | 204 | 1.00 | | — | — |
| Tacrolimus + methotrexate | 22 | 1.40 (0.81-2.40) | | — | — |
| Acute GVHD-time‡ | — | 1.25 (0.85-1.84) | .264 | 1.28 (0.87-1.90) | .213 |

CI indicates confidence interval; PTCL, peripheral T-cell lymphoma; HLA, human leukocyte antigen; BM, bone marrow; GVHD, graft-versus-host disease; and —, not applicable.

*Those who received cord blood (n = 2) or BM + PBSCs (n = 2) were excluded because of the small number of patients.

†Seven patients using other GVHD prophylaxis were excluded.

‡Acute GVHD was treated as time-dependent variable.

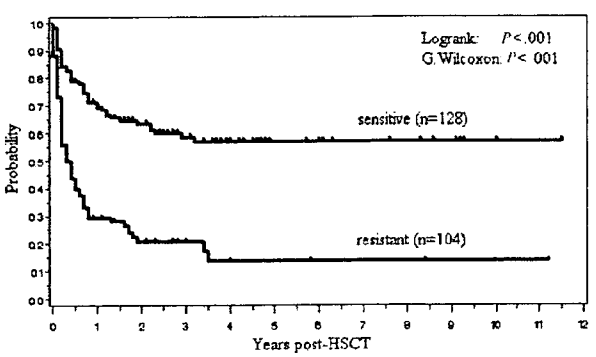


**Figure 5. The relation between overall survival and response to chemotherapy.**

## Acknowledgments

We thank the many physicians in the participating teams who contributed to this survey.

## Appendix

The following institutions contributed data to this study: Asahikawa Medical College Hospital, Hokkaido University Hospital, Sapporo Hokuyu Hospital, Akita University Hospital, Gunma Saiseikai Maebashi Hospital, Jichi Medical School Hospital, Suifu Hospital, Saitama Cancer Center Hospital, Jikei University Kashiwa Hospital, Chiba Aoba Municipal

Hospital, Tokyo Metropolitan Komagome Hospital, National Cancer Center Hospital, Keio University Hospital, Toranomon Hospital, Yokohama City University Hospital, Kanagawa Cancer Center, Tokai University Hospital, Kurobe City Hospital, Kanazawa University Hospital, Ishikawa Prefectural Central Hospital, Nagoya City University Hospital, Japanese Red Cross Nagoya First Hospital, Nagoya Daini Red Cross Hospital, Meitetsu Hospital, JA Aichi Showa Hospital, Kyoto University Hospital, Kyoto Prefectural University of Medicine Hospital, Osaka University Hospital, Osaka City University Hospital, Kansai Medical University Hospital, Kinki University Hospital, Osaka Medical Center for Cancer and Cardiovascular Diseases, Osaka City General Hospital, Rinku General Medical Center Izumisano Hospital, Hyogo College of Medicine Hospital, Hyogo Medical Center for Adults, Okayama University Hospital, Okayama Medical Center, Shimane Prefectural Central Hospital, Takamatsu Red Cross Hospital, Ehime Prefectural Central Hospital, University of Occupational and Environmental Health Hospital, Kitakyushu Municipal Medical Center, Kyushu Cancer Center, Kokura Memorial Hospital, Fukuoka University Hospital, Hamanomachi Hospital, Harasanshin Hospital, Saga Prefectural Hospital Koseikan, Sasebo Municipal General Hospital, Miyazaki Prefectural Miyazaki Hospital, Imamura Bun-in Hospital, and Ryukyu University Hospital.

## References


1. Philip T, Guglielmi C, Hagenbeek A, et al. Autologous bone marrow transplantation as compared with salvage chemotherapy in relapses of chemotherapy-sensitive non-Hodgkin's lymphoma. N Engl J Med. 1995;333:1540-1545.
2. Milpied N, Deconinck E, Gaillard F, et al. Initial treatment of aggressive lymphoma with high-dose chemotherapy and autologous stem-cell support. N Engl J Med. 2004;350:1287-1295.
3. Horning SJ, Negrin RS, Hoppe RT, et al. High-dose therapy and autologous bone marrow transplantation for follicular lymphoma in first complete or partial remission: results of a phase II clinical trial. Blood. 2001;97:404-409.
4. Dhedin N, Giraudier S, Gaulard P, et al. Allogeneic bone marrow transplantation in aggressive non-Hodgkin's lymphoma (excluding Burkitt and lymphoblastic lymphoma): a series of 73 patients from the SFGM database. Br J Haematol. 1999; 107:154-161.
5. van Besien KW, Mehra RC, Giralt SA, et al. Allogeneic bone marrow transplantation for poor-prognosis lymphoma: response, toxicity and survival depend on disease histology. Am J Med. 1996;100:299-307.
6. van Besien K, Thall P, Korbling M, et al. Allogeneic transplantation for recurrent or refractory non-Hodgkin's lymphoma with poor prognostic features after conditioning with thiotepa, busulfan, and cyclophosphamide: experience in 44 consecutive patients. Biol Blood Marrow Transplant. 1997;3:150-156.
7. van Besien K, Sobocinski KA, Rowlings PA, et al. Allogeneic bone marrow transplantation for low-grade lymphoma. Blood. 1998;92:1832-1836.
8. Peniket AJ, Ruiz de Elvira MC, Taghipour G, et al. An EBMT registry matched study of allogeneic stem cell transplants for lymphoma: allogeneic transplantation is associated with a lower relapse rate but a higher procedure-related mortality rate than autologous transplantation. Bone Marrow Transplant. 2003;31:667-678.
9. Levine JE, Harris RE, Loberiza FR Jr, et al. A comparison of allogeneic and autologous bone marrow transplantation for lymphoblastic lymphoma. Blood. 2003;101:2476-2482.
10. van Besien K, Loberiza FR Jr, Bajorunaite R, et al. Comparison of autologous and allogeneic hematopoietic stem cell transplantation for follicular lymphoma. Blood. 2003;102:3521-3529.
11. Forrest DL, Thompson K, Nevill TJ, Couban S, Fernandez LA. Allogeneic hematopoietic stem cell transplantation for progressive follicular lymphoma. Bone Marrow Transplant. 2002;29:973-978.
12. Berdeja JG, Jones RJ, Zahurak ML, et al. Allogeneic bone marrow transplantation in patients with sensitive low-grade lymphoma or mantle cell lymphoma. Biol Blood Marrow Transplant. 2001;7: 561-567.
13. Stein RS, Greer JP, Goodman S, et al. Limited efficacy of intensified preparative regimens and autologous transplantation as salvage therapy in high-grade non-Hodgkin's lymphoma. Leuk Lymphoma. 2001;40:521-528.
14. Khouri IF, Lee MS, Romaguera J, et al. Allogeneic hematopoietic transplantation for mantle-cell lymphoma: molecular remissions and evidence of graft-versus-malignancy. Ann Oncol. 1999;10: 1293-1299.
15. Zaja F, Russo D, Silvestri F, et al. Retrospective analysis of 23 cases with peripheral T-cell lymphoma, unspecified: clinical characteristics and outcome. Haematologica. 1997;82:171-177.
16. Makita M, Maeda Y, Takenaka K, et al. Successful treatment of progressive NK cell lymphoma with allogeneic peripheral stem cell transplantation followed by early cyclosporine tapering and donor leukocyte infusions. Int J Hematol. 2002;76:94-97.
17. Khouri IF, Lee MS, Saliba RM, et al. Nonablative allogeneic stem-cell transplantation for advanced/recurrent mantle-cell lymphoma. J Clin Oncol. 2003;21:4407-4412.
18. Khouri IF, Saliba RM, Giralt SA, et al. Nonablative allogeneic hematopoietic transplantation as adoptive immunotherapy for indolent lymphoma: low incidence of toxicity, acute graft-versus-host disease, and treatment-related mortality. Blood. 2001;98:3595-3599.
19. Tanimoto TE, Kusumi E, Hamaki T, et al. High complete response rate after allogeneic hematopoietic stem cell transplantation with reduced-intensity conditioning regimens in advanced malignant lymphoma. Bone Marrow Transplant. 2003;32:131-137.
20. Bierman PJ. Allogeneic bone marrow transplantation for lymphoma. Blood Rev. 2000;14:1-13.
21. Jaffe ES, Harris NL, Stein H, et al, eds. World Health Organization Classification of Tumors. Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues. Lyon, France: IARC Press; 2001.
22. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. Bone Marrow Transplant. 1995;15:825-828.
23. Shulman HM, Sullivan KM, Weiden PL, et al. Chronic graft-versus-host syndrome in man. A long-term clinicopathologic study of 20 Seattle patients. Am J Med. 1980;69:204-217.
24. Kaplan EL, Meier P. Nonparametric estimation from incomplete observations. Am Stat Assoc J. 1958;457-481.
25. Akpek G, Ambinder RF, Piantadosi S, et al. Long-term results of blood and marrow transplantation for Hodgkin's lymphoma. J Clin Oncol. 2001;19: 4314-4321.
26. Bortin MM, Kay HE, Gale RP, Rimm AA. Factors associated with interstitial pneumonitis after bone-marrow transplantation for acute leukaemia. Lancet. 1982;1:437-439.
27. Weiner RS, Bortin MM, Gale RP, et al. Interstitial pneumonitis after bone marrow transplantation. Assessment of risk factors. Ann Intern Med. 1986;104:168-175.
28. Ratanatharathorn V, Uberti J, Karanes C, et al. Prospective comparative trial of autologous versus allogeneic bone marrow transplantation in patients with non-Hodgkin's lymphoma. Blood. 1994;84:1050-1055.
29. Chopra R, Goldstone AH, Pearce R, et al. Autologous versus allogeneic bone marrow transplantation for non-Hodgkin's lymphoma: a case-controlled analysis of the European Bone Marrow Transplant Group Registry data. J Clin Oncol. 1992;10:1690-1695.
30. Freytes CO, Loberiza FR, Rizzo JD, et al. Myeloablative allogeneic hematopoietic stem cell transplantation in patients who experience relapse after autologous stem cell transplantation for lymphoma: a report of the International Bone Marrow Transplant Registry. Blood. 2004;104: 3797-3803.
31. Izutsu K, Kanda Y, Ohno H, et al. Unrelated bone marrow transplantation for non-Hodgkin lymphoma: a study from the Japan Marrow Donor Program. Blood. 2004;103:1955-1960.
32. Yanada M, Emi N, Naoe T, et al. Tacrolimus instead of cyclosporine used for prophylaxis against graft-versus-host disease improves outcome after hematopoietic stem cell transplantation from unrelated donors, but not from HLA-identical sibling donors: a nationwide survey conducted in Japan. Bone Marrow Transplant. 2004;34:331-337.
33. de Lima M, Couriel D, Thall PF, et al. Once-daily intravenous busulfan and fludarabine: clinical and pharmacokinetic results of a myeloablative, reduced-toxicity conditioning regimen for allogeneic stem cell transplantation in AML and MDS. Blood. 2004;104:857-864.
34. Russell JA, Tran HT, Quinlan D, et al. Once-daily intravenous busulfan given with fludarabine as conditioning for allogeneic stem cell transplantation: study of pharmacokinetics and early clinical outcomes. Biol Blood Marrow Transplant. 2002;8: 468-476.