chronic GVHD group" at the time of transplantation and then transferred to the "limited chronic GVHD group" or to the "extensive chronic GVHD group" at the onset of the maximum grade of chronic GVHD. The variables considered were the age group of the recipient (50 years or younger or greater than 50 years at transplantation); sex of the recipient (female or male); disease status prior to transplantation (complete remission, disease status other than complete remission, or unknown); intensity of conditioning regimen (myeloablative, reduced-intensity, or unclassifiable); type of GVHD prophylaxis (cyclosporine-based, tacrolimus-based, or others); type of donor (HLA-matched-related donor, HLA-mismatched-related donor, unrelated donor for bone marrow, or unrelated cord blood); time from diagnosis to transplantation (within 6 months, longer than 6 months, or unknown); and year of transplantation (1995–2002 or 2003–2005). We classified the intensity of conditioning regimen as myeloablative or reduced-intensity based on the working definition by Center for International Blood and Marrow Transplant Research if data on dosage of agents and total-body irradiation (TBI) used in the conditioning regimen were available.[29] For 110 patients for whom such information was not fully available, we used the information on conditioning intensity (myeloablative or reduced-intensity) reported by treating clinicians. The cutoff points for year of transplantation were chosen such that we could make optimal use of the data with a proviso that the smaller group contained at least 30% of patients. In the analysis of the effect of chronic GVHD, the prior history of grade 2 to 4 acute GVHD was also added to the multivariable models. We also assessed the interaction between acute GVHD and the intensity of conditioning regimen in the

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multivariable models. Only factors with a p value of less than 0.10 in univariable analysis were included in the multivariable models. Additionally, the heterogeneities of the effects of grade 1-2 or grade 3-4 acute GVHD on overall survival according to background transplant characteristics were evaluated by the forest plots stratified by variables included in the regression analyses. Furthermore, landmark analysis treating the development of acute GVHD as a time-fixed covariate was performed to confirm the results of analyses treating the occurrence of acute GVHD as a time-varying covariate; the landmark day was set at day 68 after transplantation, the date until when more than 95% of patients developed acute GVHD.

Results were expressed as hazard ratios and their 95% confidence intervals (CI). All tests were two-sided, and a p value of less than 0.05 was considered to indicate statistical significance. All statistical analyses were performed with STATA version 11 (Stata Corp., College Station, TX) software.

Results

Characteristics of patients and transplants

Characteristics of the patients and transplants are shown in Table 1. Most of the patients received transplants at the age of 41–60 years (median, 51 years). The disease status at transplantation was mainly defined as other than complete remission. The intensity of conditioning regimen was classified as myeloablative in 102 (35%) patients and reduced-intensity in 128 (44%) patients; the remaining 64 (22%) patients were reported to receive cyclophosphamide plus TBI in 16,

busulfan plus cyclophosphamide in 15, busulfan plus melphalan in 1, purine analogue-containing regimen in 6, and other TBI-based regimens in 26, although the intensity of these regimens was considered unclassifiable due to lack of dosage information. Cyclosporine-based prophylaxis against GVHD was used in more than half of patients. Patients underwent transplantation using HLA-matched related donor in 132 (45%), HLA-mismatched related donor in 31 (11%), unrelated bone marrow donor in 82 (28%), and unrelated cord blood unit in 49 (17%). Half of the patients received transplants within 6 months of diagnosis. The median time of follow-up among the survivors was 42.8 months (range, 1.5–102.3).

The effect of acute GVHD on overall survival

The median onset day of acute GVHD of any grade after transplantation was 24.5 (range, 5–133). Acute GVHD of grades 1–4, 2–4, and 3–4 occurred in 202 (69%), 150 (51%), and 65 patients (22%), respectively. The effect of acute GVHD on overall survival was evaluated using semi-landmark plots with reference to the following three categories: no acute GVHD, grade 1–2 acute GVHD, and grade 3–4 acute GVHD (Figure 1A). The impact of grade 1–2 or grade 3–4 acute GVHD on overall survival was also evaluated by forest plots stratified by background characteristics of patients and transplants (Figure 2). These analyses revealed that development of grade 1–2 acute GVHD was consistently associated with higher overall survival compared with the absence of acute GVHD, while occurrence of grade 3–4 acute GVHD was consistently associated with lower overall survival, except that adverse impact of grade 3–4 acute GVHD was not observed in the subgroups of patients who received transplants from an HLA-matched related or HLA-mismatched related donor. Multivariable analysis treating an occurrence of acute GVHD as a time-dependent covariate also confirmed the positive impact of grade 1–2 acute GVHD (HR, 0.65; 95% CI, 0.45–0.93; p=0.018), and adverse impact of grade 3–4 acute GVHD on overall survival (HR, 1.64; 95% CI, 1.10–2.42; p=0.014) (Table 2). Patients who received reduced-intensity conditioning and myeloablative conditioning had similar rates of overall survival by both univariable (HR of reduced-intensity versus myeloablative transplant 1.19; 95% CI, 0.85-1.68; p=0.318) and multivariable analysis (HR 0.95; 95% CI, 0.61-1.47; p=0.814). There was no interaction effect between conditioning intensity and grade 1-2 (p=0.704) or grade 3-4 acute GVHD (p=0.891) on overall survival. The effect of each grade of acute GVHD on overall survival was additionally evaluated. It showed that only grade 2 acute GVHD was associated with superior overall survival, while only grade 4 acute GVHD was associated with inferior survival (Supplemental Table 1). In the landmark analysis treating an occurrence of acute GVHD as a time-fix covariate, consistent results were obtained for patients who survived at least 68 days (landmark day), although the adverse impact of grade 3–4 acute GVHD on overall survival became no longer significant (Supplemental Table 2).

The effects of acute GVHD on disease-associated and treatment-related mortality

We next evaluated the effects of acute GVHD on disease-associated and

treatment-related mortality (Figure 1B, 1C). Disease-associated mortality was defined as cumulative incidence of death directly attributable to relapse or progression of ATL, while treatment-related mortality was calculated as cumulative incidence of any death not included in disease-associated deaths. Multivariable analysis revealed that disease-associated mortality was lower in the presence of grade 1–2 and grade 3–4 acute GVHD compared with the absence of acute GVHD (grade 1–2 acute GVHD: HR, 0.54; 95% CI, 0.32–0.92; p=0.023; and grade 3–4 acute GVHD: HR, 0.44; 95% CI, 0.22–0.90; p=0.024) (Table 2), and each grade of acute GVHD showed consistent inverse association with disease-associated mortality (Supplemental Table 1). Although the risk of treatment-related mortality was not higher in the presence of grade 1-2 acute GVHD, development of grade 3–4 acute GVHD was significantly associated with higher treatment-related mortality compared with the absence of acute GVHD (HR, 3.50; 95% CI, 2.01–6.11; p<0.001) (Table 2). Patients undergoing reduced-intensity transplantation and those undergoing myeloablative transplantation had similar risks of disease-associated death (HR, 0.99; 95% CI, 0.46-2.13; p=0.975) and treatment-related death (HR, 0.98; 95% CI, 0.60-1.59; p=0.928) by multivariable analysis. There was no interaction effect between conditioning intensity and grade 1-2 or grade 3-4 acute GVHD on disease-associated mortality and treatment-related mortality. Of 95 patients who experienced treatment-related deaths, 27 succumbed to infectious complications: bacterial in 13, viral in 7 (including 3 cases of CMV disease), viral and bacterial in 1, fungal in 5, and no specific organism reported in 1. The proportions of patients who died of infectious complication among those without

acute GVHD (n=92), those with grade 1-2 (n=137), and those with grade 3-4 acute GVHD (n=65) were 4%, 9%, and 17%, respectively (Supplemental Table 3). By multivariable analysis, development of grade 3-4 acute GVHD was significantly associated with higher risk of death related to infection (HR, 4.74; 95% CI, 1.51-14.8; p=0.008), while the adverse influence on the infection-related deaths was less evident in the presence of grade 1-2 acute GVHD (HR, 2.17; 95% CI, 0.72-6.56; p=0.169).

The effects of chronic GVHD on overall survival and mortality

Chronic GVHD was evaluated in 183 patients who survived at least 100 days after transplantation. The median day of chronic GVHD occurrence after transplantation was 116 (range, 100–146). Limited and extensive chronic GVHD occurred in 29 (16%) and 63 patients (34%), respectively. Semi-landmark plots were constructed to illustrate the effects of chronic GVHD on overall survival, disease-associated mortality, and treatment-related mortality with reference to the following subgroups: no chronic GVHD, limited chronic GVHD, and extensive chronic GVHD (Figure 3). In multivariable analysis treating an occurrence of chronic GVHD as a time-dependent covariate, neither overall survival nor disease-associated mortality was significantly associated with severity of chronic GVHD, while treatment-related mortality was higher in the presence of extensive chronic GVHD (HR, 2.75; 95% CI, 1.34–5.63; p=0.006) compared with the absence of chronic GVHD (Table 3). The proportions of patients who died of infectious complication among those without chronic GVHD (n=91), those with limited chronic GVHD (n=29), and those with extensive chronic GVHD (n=63)

were 7%, 10%, and 8%, respectively. In multivariable analysis, no statistically significant association was found between infection-related death and the occurrence of either limited (p=0.289) or extensive GVHD (p=0.836).

Discussion

To our knowledge, this is the largest retrospective study to analyze the impact of acute and chronic GVHD on clinical outcomes including overall survival, disease-associated mortality, and treatment-related mortality after allogeneic HCT for ATL. In the present study, the occurrence of both grade 1–2 and grade 3–4 acute GVHD was associated with lower disease-associated mortality compared with the absence of acute GVHD. However, positive effect of GVHD on reduced disease-associated mortality was counterbalanced by increased treatment-related mortality among patients who developed severe acute GVHD, and an overall beneficial effect on survival was observed only with the development of mild-to-moderate acute GVHD. In contrast to acute GVHD, no beneficial effect was observed in association with the development of chronic GVHD, although the point estimate of the hazard ratio comparing limited chronic GVHD versus the absence of chronic GVHD suggested the trend toward a reduced risk of disease-associated deaths in the limited chronic GVHD group.

Our present findings are in contrast to the previous reports showing the beneficial effects of chronic GVHD rather than acute GVHD on the prevention of disease recurrence after allogeneic HCT. It is less likely that the particular characteristics of chronic GVHD in patients with ATL biased the results, because the incidence rate and median onset day of chronic GVHD in our cohort were

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similar to those reported in previous studies evaluating the incidence of chronic GVHD among Japanese patients, most of whom had received allogeneic HCT for myeloid neoplasms or acute lymphoblastic leukemia.[30-32] Conceivably, the rapid tempo of disease recurrence of ATL might be such that chronic GVHD is less potent in terms of harnessing clinically relevant graft-versus-leukemia responses as compared with acute GVHD. However, the results of our analysis regarding the effect of chronic GVHD should be interpreted with caution because the number of patients evaluable for chronic GVHD were relatively small in our study for providing sufficient statistical power. The effect of chronic GVHD on outcomes after HCT for ATL should be further explored in a larger cohort.

The occurrence of GVHD has been shown to exert a potent graft-versus-leukemia effect in terms of reducing relapse incidence in acute leukemia or chronic myeloid leukemia.[33,34] On the other hand, multiple studies have documented a correlation between GVHD in its acute or chronic form and treatment-related mortality. In a study of patients undergoing HLA-identical sibling HCT for chronic myeloid leukemia, the overall beneficial effect on long-term survival was demonstrated only in a group of patients who developed grade 1 acute GVHD or limited chronic GVHD.[33] In another study of HLA-identical sibling HCT for leukemia using cyclosporine and methotrexate as GVHD prophylaxis, a benefit of mild GVHD was only seen in high-risk patients but not in standard-risk patients. Therefore, the therapeutic window between decreased relapse incidence and increased transplant-related mortality in association with the development of GVHD has been considered to be very narrow.[34]

With regard to the effectiveness of allogeneic HCT for ATL, it is also of note here that post-transplant eradication of ATL cells can be achieved without the use of high-dose chemoradiotherapy: patients who received a transplant with reduced-intensity conditioning had survival outcomes similar to those who received a transplant with myeloablative conditioning in our study. Intriguingly, several small cohort studies exhibited that abrupt discontinuation of immunosuppressive agents resulted in disappearance or reduction in the tumor burden in allografted patients with ATL. In some cases, remission of ATL was observed along with the development of GVHD.[19,20,22] Taken together with the findings of this study, it is suggested that ATL is particularly susceptible to immune modulation following allogeneic HCT. To clarify the presence of such "graft-versus-ATL" effect, further investigations are needed to assess the efficacy of donor lymphocyte infusion or withdrawal of immunosuppressive agents on relapse after transplantation.

Of the HTLV-I gene products, Tax is a dominant target of HTLV-I-specific cytotoxic T lymphocytes. The vigorous Tax-specific cytotoxic T-cell responses were demonstrated in recipients who obtained complete remission after allogeneic HCT for ATL, suggesting that "graft-versus-HTLV-I" responses might contribute to the eradication of ATL cells.[35,36] However, Tax is generally undetectable or present in very low levels in primary ATL cells.[37,38] In addition, small amounts of HTLV-I provirus can be detected in peripheral blood of recipients who attained long-term remission of ATL, even after HCT from HTLV-I-negative donors.[39,40] These findings suggest that "graft-versus-ATL" effect can be harnessed without complete elimination of HTLV-I. It is also

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important to note that allogeneic HCT is emerging as an effective treatment option for other mature T-cell neoplasms not related to HTLV-I, such as mycosis fungoides/Sézary syndrome and various types of aggressive peripheral T-cell lymphomas.[41,42] These observations raised the possibility that the common targets for alloimmune responses might exist across a spectrum of malignant T-cell neoplasms including ATL. The minor histocompatibility antigens or tumor-specific antigens can be other targets of alloimmune anti-ATL effect.[43-45] Therefore, the elucidation of the mechanism underlying an immunologic eradication of primary ATL cells may lead to a new strategy for improving outcomes of allogeneic HCT not only for ATL but for other intractable T-cell neoplasms.

This study has several limitations. First, acute GVHD might be intentionally induced for some patients considered at high risk of relapse by treating clinicians. Secondly, the information on the day when each grade of GVHD occurred was not available. Therefore, we treated the development of acute and chronic GVHD in their worst severity as a time-varying covariate. To validate the results, we also performed the landmark analysis and obtained consistent results. Thirdly, the relatively small number of patients with chronic GVHD might mask or bias the effect of chronic GVHD on outcomes. Lastly, the effect of multiple testing should be taken into account for the interpretation of the secondary endpoints.

In conclusion, the development of acute GVHD was associated with lower disease-associated mortality after allogeneic HCT for ATL as compared with the absence of acute GVHD. However, improved survival can be expected only among a group of patients who developed mild-to-moderate acute GVHD

because those who developed severe acute GVHD were at high risk of treatment-related mortality. New strategies that enhance the allogeneic anti-ATL effect without exacerbating GVHD are required to improve the outcomes of patients undergoing allogeneic HCT for ATL.

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The views expressed in this report are those of authors and do not indicate the views of the JSHCT, JMDP, or JCBBN.

Authorship

Contribution: T.I. and T.U. designed the research and organized the project; M. Hishizawa, J.K., T.I., and T.U. reviewed data, analyzed data, and wrote the paper; J.K., T.I., and K.M. performed statistical analysis; Y.A., R.S., and H.S. collected data from JSHCT; T.K. and Y. Morishima collected data from JMDP; T. N-I. and S. Kato collected data from JCBBN; A.U., S.T., T.E., Y. Moriuchi., R.T., F.K., Y. Miyazaki., M.M., K.N., M. Hara, M.T., S.Kai., and J.O. interpreted data, reviewed and approved final manuscript.

T.U., the senior author, was deceased during the preparation of this manuscript.

In addition to authors, other members who contributed data on allogeneic HSCT for ATL to JSHCT, JMDP, and JCBBN are listed in the supplemental Appendix.

Conflict-of-interest disclosure

The authors declare no competing financial interests.

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References

1. Uchiyama T, Yodoi J, Sagawa K, Takatsuki K, Uchino H. Adult T-cell leukemia: clinical and hematologic features of 16 cases. *Blood.* 1977; 50(3):481-492.

2. Uchiyama T. Human T cell leukemia virus type I (HTLV-I) and human diseases. *Annu Rev Immunol.* 1997; 15:15-37.

3. Verdonck K, Gonzalez E, van Dooren S, Vandamme AM, Vanham G, Gotuzzo E. Human T-lymphotropic virus 1: recent knowledge about an ancient infection. *Lancet Infect Dis.* 2007; 7(4):266-281.

4. Matsuoka M, Jeang KT. Human T-cell leukaemia virus type 1 (HTLV-1) infectivity and cellular transformation. *Nat Rev Cancer.* 2007; 7(4):270-280.

5. Arisawa K, Soda M, Endo S, et al. Evaluation of adult T-cell leukemia/lymphoma incidence and its impact on non-Hodgkin lymphoma incidence in southwestern Japan. *Int J Cancer.* 2000; 85(3):319-324.

6. Shimoyama M. Diagnostic criteria and classification of clinical subtypes of adult T-cell leukaemia-lymphoma. A report from the Lymphoma Study Group (1984-87). *Br J Haematol.* 1991; 79(3):428-437.

7. Yamada Y, Tomonaga M, Fukuda H, et al. A new G-CSF-supported combination chemotherapy, LSG15, for adult T-cell leukaemia-lymphoma: Japan Clinical Oncology Group Study 9303. *Br J Haematol.* 2001; 113(2):375-382.

8. Tsukasaki K, Utsunomiya A, Fukuda H, et al. VCAP-AMP-VECP compared with biweekly CHOP for adult T-cell leukemia-lymphoma: Japan Clinical Oncology Group Study JCOG9801. *J Clin Oncol.* 2007; 25(34):5458-5464.

9. Bazarbachi A, Ghez D, Lepelletier Y, et al. New therapeutic approaches for adult T-cell leukaemia. *Lancet Oncol.* 2004; 5(11):664-672.

10. Kchour G, Tarhini M, Kooshyar MM, et al. Phase 2 study of the efficacy and safety of the combination of arsenic trioxide, interferon alpha, and zidovudine in newly diagnosed chronic adult T-cell leukemia/lymphoma (ATL). *Blood.* 2009; 113(26): 6528-6532.

11. Yamamoto K, Utsunomiya A, Tobinai K, et al. Phase I study of KW-0761, a defucosylated humanized anti-CCR4 antibody, in relapsed patients with adult T-cell leukemia-lymphoma and peripheral T-cell lymphoma. *J Clin Oncol.* 2010; 28(9):1591-1598.

12. Jabbour M, Tuncer H, Castillo J, et al. Hematopoietic SCT for adult T-cel leukemia/lymphoma: a review. *Bone Marrow Transplant.* 2011; 46(8):1039-1044.

13. Utsunomiya A, Miyazaki Y, Takatsuka Y, et al. Improved outcome of adult T cell leukemia/lymphoma with allogeneic hematopoietic stem cell transplantation. *Bone Marrow Transplant.* 2001; 27(1):15-20.

14. Kami M, Hamaki T, Miyakoshi S, et al. Allogeneic haematopoietic stem cell transplantation for the treatment of adult T-cell leukaemia/lymphoma. *Br J Haematol.* 2003; 120(2):304-309.

15. Fukushima T, Miyazaki Y, Honda S, et al. Allogeneic hematopoietic stem cell transplantation provides sustained long-term survival for patients with adult T-cell leukemia/lymphoma. *Leukemia.* 2005; 19(5):829-834.

16. Okamura J, Utsunomiya A, Tanosaki R, et al. Allogeneic stem-cell transplantation with reduced conditioning intensity as a novel immunotherapy and antiviral therapy for adult T-cell leukemia/lymphoma. *Blood.* 2005; 105(10):4143-4145.

17. Nakase K, Hara M, Kozuka T, Tanimoto K, Nawa Y. Bone marrow transplantation from unrelated donors for patients with adult T-cell leukaemia/lymphoma. *Bone Marrow Transplant.* 2006; 37(1):41-44.

18. Kato K, Kanda Y, Eto T, et al. Allogeneic bone marrow transplantation from unrelated human T-cell leukemia virus-I-negative donors for adult T-cell leukemia/lymphoma: retrospective analysis of data from the Japan Marrow Donor Program. *Biol Blood Marrow Transplant.* 2007; 13(1):90-99.

19. Yonekura K, Utsunomiya A, Takatsuka Y, et al. Graft-versus-adult T-cell

leukemia/lymphoma effect following allogeneic hematopoietic stem cell transplantation. *Bone Marrow Transplant.* 2008; 41(12):1029-1035.

20. Shiratori S, Yasumoto A, Tanaka J, et al. A retrospective analysis of allogeneic hematopoietic stem cell transplantation for adult T cell leukemia/lymphoma (ATL): clinical impact of graft-versus-leukemia/lymphoma effect. *Biol Blood Marrow Transplant.* 2008;14(7):817-823.

21. van Besien KW, de Lima M, Giralt SA, et al. Management of lymphoma recurrence after allogeneic transplantation: the relevance of graft-versus-lymphoma effect. *Bone Marrow Transplant.* 1997; 19(10):977-982.

22. Tanosaki R, Uike N, Utsunomiya A, et al. Allogeneic hematopoietic stem cell transplantation using reduced-intensity conditioning for adult T cell leukemia/lymphoma: impact of antithymocyte globulin on clinical outcome. *Biol Blood Marrow Transplant.* 2008; 14(6):702-708.

23. Hishizawa M, Kanda J, Utsunomiya A, et al. Transplantation of allogeneic hematopoietic stem cells for adult T-cell leukemia: a nationwide retrospective study. *Blood.* 2010; 116(8):1369-1376.

24. Przepiorka D, Weisdorf D, Martin P, et al. 1994 Consensus Conference on Acute GVHD Grading. *Bone Marrow Transplant.* 1995; 15(6):825-828.

25. 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(3):250-259.

26. Gooley TA, Leisenring W, Crowley J, Storer BE. Estimation of failure probabilities in the presence of competing risks: new representations of old estimators. *Stat Med.* 1999; 18(6):695-706.

27. Fine JP, Gray RJ. A proportional hazards model for subdistribution of a competing risk. *J Am Stat Assoc.* 1999; 94:496-509.

28. Cortese G, Andersen P. Competing risks and time-dependent covariates. *Biom J.*

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2010; 52(1):138-158.

29. Giralt S, Ballen K, Rizzo D, et al. Reduced-intensity conditioning regimen workshop: defining the dose spectrum. Report of a workshop convened by the Center for International Blood and Marrow Transplant Research. *Biol Blood Marrow Transplant.* 2009; 15(3): 367-369.

30. Atsuta Y, Suzuki R, Yamamoto K, et al. Risk and prognostic factors for Japanese patients with chronic graft-versus-host disease after bone marrow transplantation. *Bone Marrow Transplant.* 2006; 37(3):289-296.

31. Ozawa S, Nakaseko C, Nishimura M, et al. Chronic graft-versus-host disease after allogeneic bone marrow transplantation from an unrelated donor: incidence, risk factors and association with relapse. A report from the Japan Marrow Donor Program. *Br J Haematol.* 2007; 137(2):142-151.

32. Nagafuji K, Matsuo K, Teshima T, et al. Peripheral blood stem cell versus bone marrow transplantation from HLA-identical sibling donors in patients with leukemia: a propensity score-based comparison from the Japanese Society for Hematopoietic Stem Cell Transplantation registry. *Int J Hematol.* 2010; 91(5):855-864.

33. Gratwohl A, Brand R, Apperley J, et al. Graft-versus-host disease and outcome in HLA-identical sibling transplantations for chronic myeloid leukemia. *Blood.* 2002; 100(12):3877-3886.

34. Kanda Y, Izutsu K, Hirai H, et al. Effect of graft-versus-host disease on the outcome of bone marrow transplantation from an HLA-identical sibling donor using GVHD prophylaxis with cyclosporin A and methotrexate. *Leukemia.* 2004; 18(5):1013-1019.

35. Harashima N, Kurihara K, Utsunomiya A, et al. Graft-versus-Tax response in adult T-cell leukemia patients after hematopoietic stem cell transplantation. *Cancer Res.* 2004; 64(1):391-399.

36. Tanaka Y, Nakasone H, Yamazaki R, et al. Single-cell analysis of T-cell repertoire of HTLV-1 Tax-specific cytotoxic T cells in allogeneic transplant recipients with adult T-cell leukemia/lymphoma. *Cancer Res.* 2010; 70(15):6181-6192.

37. Franchini G, Wong-Staal F, Gallo RC. Human T-cell leukemia virus (HTLV-I) transcripts in fresh and cultured cells of patients with adult T-cell leukemia. *Proc Natl Acad Sci U S A.* 1984; 81(19):6207-6211.

38. Kinoshita T, Shimoyama M, Tobinai K, et al. Detection of mRNA for the tax1/rex1 gene of human T-cell leukemia virus type I in fresh peripheral blood mononuclear cells of adult T-cell leukemia patients and viral carriers by using the polymerase chain reaction. *Proc Natl Acad Sci U S A.* 1989; 86(14):5620-5624.

39. Choi I, Tanosaki R, Uike N, et al. Long-term outcomes after hematopoietic SCT for adult T-cell leukemia/lymphoma: results of prospective trials. *Bone Marrow Transplant.* 2011; 46(1):116-118.

40. Yamasaki R, Miyazaki Y, Moriuchi Y, et al. Small number of HTLV-1-positive cells frequently remains during complete remission after allogeneic hematopoietic stem cell transplantation that are heterogeneous in origin among cases with adult T-cell leukemia/lymphoma. *Leukemia.* 2007; 21(6):1212-1217.

41. Le Gouill S, Milpied N, Buzyn A, et al. Graft-versus-lymphoma effect for aggressive T-cell lymphomas in adults: a study by the Société Française de Greffe de Moëlle et de Thérapie Cellulaire. *J Clin Oncol.* 2008; 26(14):2264-2271.

42. Duarte RE, Canals C, Onida F, et al. Allogeneic hematopoietic cell transplantation for patients with mycosis fungoides and Sézary syndrome: a retrospective analysis of the Lymphoma Working Party of the European Group for Blood and Marrow Transplantation. *J Clin Oncol.* 2010; 28(29):4492-4499.

43. Hishizawa M, Imada K, Ishikawa T, Uchiyama T. Kinetics of proviral DNA load, soluble interleukin-2 receptor level and tax expression in patients with adult T-cell leukemia receiving allogeneic stem cell transplantation. *Leukemia.* 2004; 18(1):167-169.

44. Hishizawa M, Imada K, Sakai T, et al. Antibody responses associated with the graft-versus-leukemia effect in adult T-cell leukemia. *Int J Hematol.* 2006; 83(4):351-355.

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45. Kawahara M, Hori T, Matsubara Y, et al. Cyclin-dependent kinaselike 5 is a novel target of immunotherapy in adult T-cell leukemia. *J Immunother.* 2007; 30(5):499-505.

Figure legends

Figure 1. Semi-landmark plots illustrating the effects of acute GVHD on overall survival (A), disease-associated mortality (B), and treatment-related mortality (C).

Figure 2. Impact of the grade of acute GVHD on overall survival in each stratified category. Effects of grade 1–2 (Panel A) and grade 3–4 acute GVHD (Panel B) on overall survival are shown as forest plots. Square boxes on lines indicate hazard ratios compared with "no acute GVHD group", and horizontal lines represent the corresponding 95% confidential intervals. Abbreviations used are the same as described in the footnotes to Tables 1 and 2.

Figure 3. Semi-landmark plots illustrating impact of chronic GVHD on overall survival (A), disease-associated mortality (B), and treatment-related mortality (C).

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Table 1. Characteristics of patients and transplants

Variables	No. of patients (%) (n = 294)
Age group at transplant (years)	
<=30	7 (2)
>30-40	30 (10)
>40-50	109 (37)
>50-60	123 (42)
>60	25 (9)
Sex	
Male	158 (54)
Female	136 (46)
Disease status	
Complete remission	99 (34)
Not in complete remission	178 (61)
Unknown	17 (6)
Conditioning regimen	
Myeloablative	102 (34)
Reduced-intensity	128 (44)
Unclassifiable	64 (22)
GVHD prophylaxis	
Cyclosporine-based	195 (66)
Tacrolimus-based	94 (32)
Others	5 (2)
Source of stem cells	
Bone marrow	132 (45)
Peripheral blood	111 (38)
Bone marrow + peripheral blood	2 (1)
Cord blood	49 (17)
Type of donor*	
HLA-matched related	132 (45)
HLA-mismatched related	31 (11)
Unrelated, bone marrow	82 (28)
Unrelated, cord blood	49 (17)
Time from diagnosis to transplant	
<=6 months	141 (48)
>6 months	141 (48)
Uncertain/missing	12 (4)
Year of transplant	
1995-1999	22 (7)
2000-2002	91 (31)
2003-2005	181 (62)
Follow-up of survivors	
Median time† (range)	42.8 (1.5–102.3)

Data are numbers (%) unless specified otherwise.

Abbreviations: Cyclosporine-based, cyclosporine with or without other agents; tacrolimus-based, tacrolimus with or without other agents.

*HLA compatibility was defined according to the results of serologic or low-resolution molecular typing for HLA-A, B and DR antigens. †Data are expressed in months.

Table 2. Effect of acute GVHD on overall survival, disease-associated mortality, and treatment–related mortality after allogeneic hematopoietic cell transplantation for adult T-cell leukemia

Outcome	Univariable analysis		Multivariable analysis	
	Hazard ratio (95% CI)	P	Hazard ratio (95% CI)	P
Overall survival*				
Grade 1–2 acute GVHD vs no acute GVHD	0.60 (0.42-0.85)	0.004	0.65 (0.45-0.93)	0.018
Grade 3–4 acute GVHD vs no acute GVHD	1.38 (0.94-2.01)	0.099	1.64 (1.10-2.42)	0.014
Disease-associated mortality†				
Grade 1–2 acute GVHD vs no acute GVHD	0.47 (0.28-0.79)	0.005	0.54 (0.32-0.92)	0.023
Grade 3–4 acute GVHD vs no acute GVHD	0.41 (0.21-0.81)	0.010	0.44 (0.22-0.90)	0.024
Treatment-related mortality¶				
Grade 1–2 acute GVHD vs no acute GVHD	1.13 (0.67-1.89)	0.649	1.22 (0.72-2.07)	0.461
Grade 3–4 acute GVHD vs no acute GVHD	3.34 (1.94-5.74)	<0.001	3.50 (2.01-6.11)	<0.001

Abbreviations: GVHD, graft-versus-host disease; CI, confidence interval.

*Other significant variables were; sex of recipient, female (reference, 1.00), male (HR, 1.70; 95% CI, 1.24–2.32; p=0.001); achievement of complete remission, complete remission (reference, 1.00), status other than complete remission (HR, 2.05; 95% CI, 1.44–2.92; p<0.001), status not known, (HR, 2.21; 95% CI, 1.15–4.22; p=0.017); type of donor, HLA-matched related donor (reference, 1.00), HLA-mismatched related donor (HR, 1.71; 95% CI, 1.04–2.84; p=0.036), unrelated donor of bone marrow (HR, 1.39; 95% CI, 0.94–2.06; p=0.096), unrelated cord blood (HR, 1.86; 95% CI, 1.22–2.83; p=0.004).

†Other significant variables were; achievement of complete remission, complete remission (reference, 1.00), status other than complete remission (HR, 2.98; 95% CI, 1.62–5.47; p<0.001) , status not known, (HR, 0.96; 95% CI, 0.21–4.49; p=0.963); type of donor, HLA-matched related donor (reference, 1.00), HLA-mismatched related donor (HR, 2.14; 95% CI, 1.00–4.55; p=0.049), unrelated donor of bone marrow (HR, 1.45; 95% CI, 0.81–2.61; p=0.214), unrelated cord blood (HR, 1.25; 95% CI, 0.63–2.49; p=0.517).

¶Another significant variable was; achievement of complete remission, complete remission (reference, 1.00), status other than complete remission (HR, 1.17; 95% CI, 0.74–1.84; p=0.498), status not known, (HR, 2.31; 95% CI, 1.04–5.15; p=0.040).

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Table 3. Effect of chronic GVHD on overall survival, disease-associated mortality, and treatment-related mortality after allogeneic hematopoietic cell transplantation for adult T-cell leukemia

Outcome	Univariable analysis		Multivariable analysis	
	Hazard ratio (95% CI)	P	Hazard ratio (95% CI)	P
Overall survival*				
Limited chronic GVHD vs no chronic GVHD	0.71 (0.34-1.47)	0.353	0.72 (0.35-1.50)	0.385
Extensive chronic GVHD vs no chronic GVHD	1.45 (0.90-2.35)	0.131	1.40 (0.86-2.30)	0.176
Disease-associated mortality†				
Limited chronic GVHD vs no chronic GVHD	0.45 (0.14-1.46)	0.183	0.45 (0.14-1.44)	0.178
Extensive chronic GVHD vs no chronic GVHD	0.81 (0.39-1.67)	0.563	0.80 (0.39-1.64)	0.536
Treatment-related mortality¶				
Limited chronic GVHD vs no chronic GVHD	1.59 (0.64-3.95)	0.316	1.56 (0.63-3.87)	0.342
Extensive chronic GVHD vs no chronic GVHD	2.85 (1.41-5.77)	0.004	2.75 (1.34-5.63)	0.006

Abbreviations: GVHD, graft-versus-host disease; CI, confidence interval.

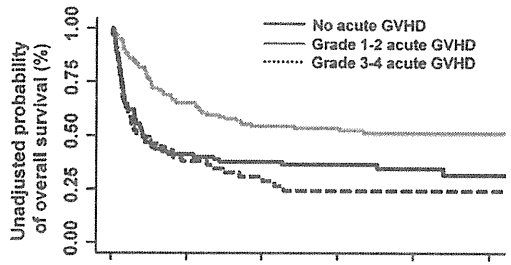
*There was no significant variable.

†There was no significant variable.

¶There was no other significant variable.

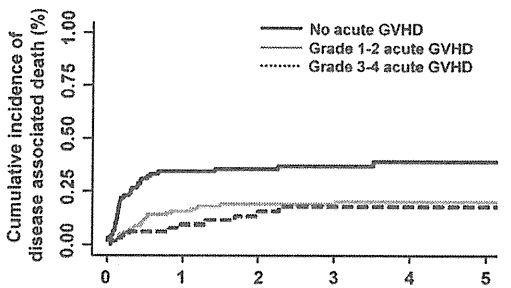
Figure 1.

A



Number at risk						
No acute GVHD	92	37	32	23	12	7
Grade 1-2 aGVHD	137	83	65	48	29	19
Grade 3-4 aGVHD	65	23	14	8	4	3

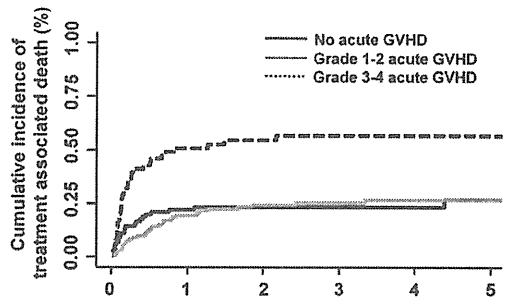
B



Number at risk						
No acute GVHD	88	36	32	23	12	7
Grade 1-2 aGVHD	134	80	65	48	29	19
Grade 3-4 aGVHD	64	23	14	8	4	3

Figure 1. (continued)

C



Number at risk						
No acute GVHD	88	36	32	23	12	7
Grade 1-2 aGVHD	134	80	65	48	29	19
Grade 3-4 aGVHD	64	23	14	8	4	3

Figure 2.

Panel A.

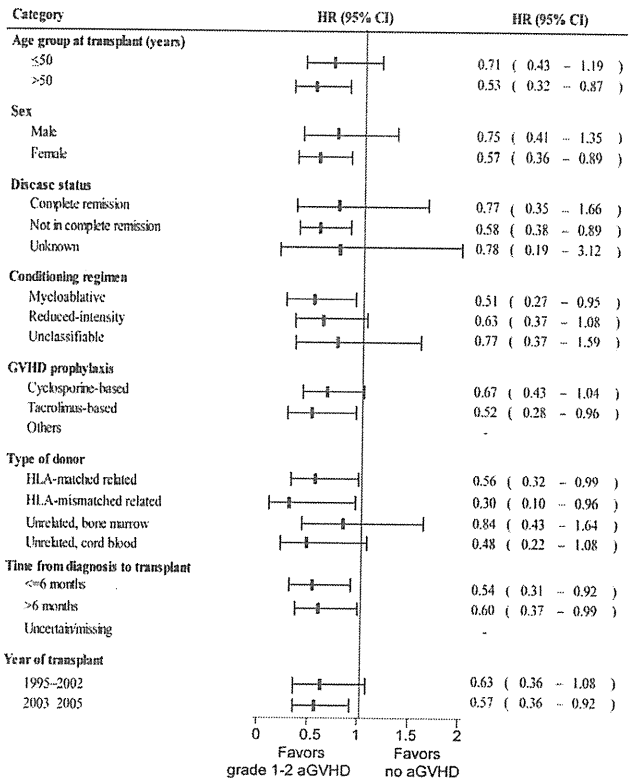


Figure 2. (continued)

Panel B.

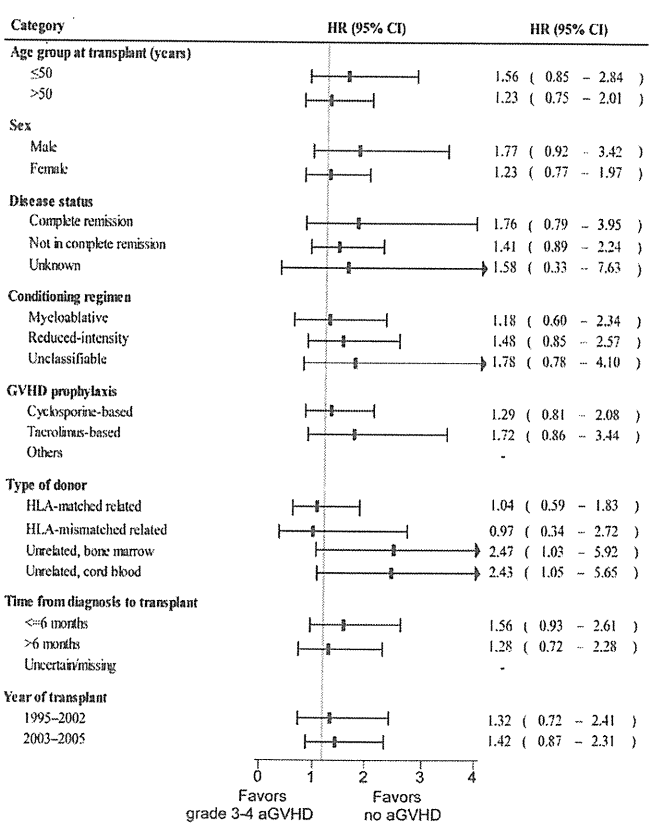
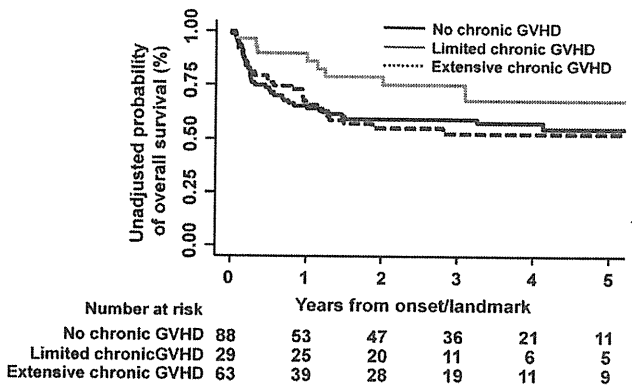


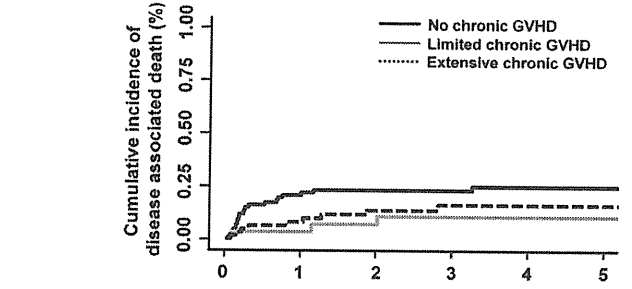
Figure 3.

A



Number at risk						
No chronic GVHD	88	53	47	36	21	11
Limited chronicGVHD	29	25	20	11	6	5
Extensive chronic GVHD	63	39	28	19	11	9

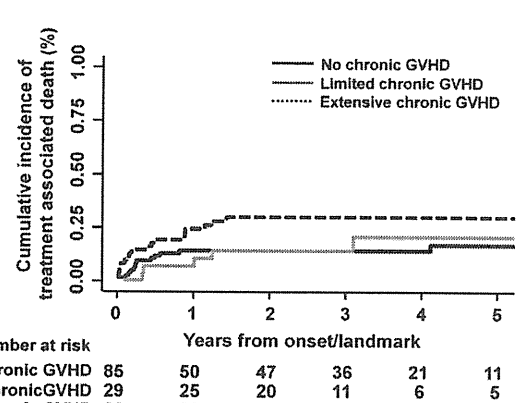
B



Number at risk						
No chronic GVHD	85	50	47	36	21	11
Limited chronicGVHD	29	25	20	11	6	5
Extensive chronic GVHD	62	38	28	19	11	9

Figure 3. (continued)

C



Number at risk						
No chronic GVHD	85	50	47	36	21	11
Limited chronicGVHD	29	25	20	11	6	5
Extensive chronic GVHD	62	38	28	19	11	9

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ORIGINAL ARTICLE

Clinical significance of subcategory and severity of chronic graft-versus-host disease evaluated by National Institutes of Health consensus criteria

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Abstract To evaluate the clinical significance of subcategory and severity of chronic graft-versus-host disease (GVHD) as defined by the National Institutes of Health (NIH) consensus criteria, we retrospectively studied 211 patients with hematologic neoplasms who survived beyond 100 days after allogeneic hematopoietic cell transplantation. Endpoints included chronic GVHD-specific survival (cGSS), duration of immunosuppressive treatment, and non-relapse mortality (NRM). A total of 96 patients fulfilled the NIH diagnostic criteria for cGVHD. In univariable analysis, patients with NIH overlap syndrome tended to exhibit lower cGSS compared to those with NIH classic cGVHD [hazard ratio (HR) = 2.76, $P = 0.060$], while patients with severe cGVHD at onset had a significantly lower cGSS compared to those with mild-to-moderate cGVHD (HR = 3.10, $P = 0.034$). The duration of immunosuppressive treatment was not significantly affected by either subcategory or severity of NIH cGVHD. In multivariable analysis treating cGVHD as a time-dependent covariate, development of overlap syndrome (HR = 3.90, $P = 0.014$) or severe cGVHD at peak worsening (HR = 6.21, $P < 0.001$) was significantly associated with higher risk of NRM compared to the absence of cGVHD. Our results suggest that both the subcategory and severity of NIH cGVHD are partly correlated with cGSS and may play a useful role in distinguishing patients at high risk for NRM, warranting validation of this approach through future prospective studies.

Keywords Hematopoietic cell transplantation · Chronic graft-versus-host disease · NIH consensus criteria

1 Introduction

Chronic graft-versus-host disease (cGVHD) remains a serious complication associated with substantial late morbidity and mortality after allogeneic hematopoietic cell transplantation (allo-HCT). In contrast to acute GVHD (aGVHD), which preferentially affects specific organs such as the skin, liver, and gastrointestinal tract, cGVHD presents with protean organ dysfunctions and various degrees of immunodeficiency that is further worsened by immunosuppressive medications used for relieving symptoms associated with GVHD [1]. Previous studies have identified a variety of factors that increase the risk of the development of cGVHD, including a prior history of aGVHD, older patient age, use of alloimmune female donors for male recipients, transplants from unrelated or human leukocyte antigen (HLA)-mismatched donors, and use of peripheral blood grafts [2–10]. In this context, clinical management of cGVHD has increasingly become more important, because recent trends in allo-HCT such as expanding applications of peripheral blood stem cell

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transplantation after reduced-intensity conditioning in older patients may increase the incidence of cGVHD [11].

Historically, aGVHD and cGVHD were distinguished based on whether immune-mediated organ dysfunction occurred within 100 days or more than 100 days after transplantation. However, accumulating experience has indicated that clinical manifestations similar to aGVHD can develop even several months after allo-HCT, while GVHD with typical features of the "chronic" form can occur as early as 2 months post-transplantation [12, 13]. Therefore, an arbitrary classification using the timing of GVHD onset is no longer considered appropriate. Another drawback in the management of cGVHD is that the grading criteria for its severity has not been standardized: it is difficult to predict the risk of GVHD-associated mortality by using historic classification that categorizes cGVHD into limited and extensive subtypes [14], because clinical severity as well as organ involvement of patients classified as having extensive cGVHD varies considerably [15–17].

To resolve these issues, the National Institutes of Health (NIH) consensus criteria were recently proposed to standardize the diagnosis and global assessment of cGVHD with a new severity scoring system based on organ-specific manifestations taking functional impact into account [18]. The NIH criteria distinguished two subcategories of cGVHD, "classic cGVHD" without features of aGVHD and "an overlap syndrome" in which characteristic features of both cGVHD and aGVHD are simultaneously present. In particular, features of aGVHD occurring beyond day 100 without manifestations of classic cGVHD are classified as "persistent", "recurrent", or "late-onset" aGVHD. Based on the number of involved organs and the severity within affected organs, each subcategory of cGVHD was graded into mild, moderate, or severe subtype. However, clinical significance of NIH cGVHD subcategory as well as their severity is not fully established, although several studies have shown their impact on overall survival, cGVHD-specific survival (cGSS), and non-relapse mortality (NRM) [19–23].

In the present study, we retrospectively evaluated patients who received allo-HCT for intractable hematologic disorders with special focus on the influences of subcategory and severity of NIH cGVHD on clinical outcomes. Since probabilities of GVHD-specific survival and discontinued immunosuppressive treatment (IST) have been most commonly used as surrogate endpoints representing the clinical resolution of cGVHD [24–26], we analyzed factors associated with these outcomes in patients who developed NIH cGVHD. We also evaluated the impact of the presence or absence of each subtype of NIH cGVHD on NRM.

2 Patients and methods

2.1 Patients

We retrospectively reviewed the medical records of 259 consecutive patients with hematologic disorders who underwent allo-HCT between January 2000 and December 2008 in our department and survived at least 100 days after transplantation. Patients were excluded if they had a history of previous allo-HCT ($n = 24$), rejected graft ($n = 4$), or relapsed before day 100 ($n = 20$); thus, a total of 211 patients were included in the present analysis. No patients received donor lymphocyte infusions before day 100. Patients with malignant hematologic neoplasms were defined as having standard-risk disease if they underwent transplantation in first complete remission or without prior chemotherapy, while those who underwent transplantation in any other status were classified as having high-risk disease. Patients with aplastic anemia were considered to have standard-risk disease. This study was approved by the Ethics Committee of Kyoto University Graduate School of Medicine. Written informed consent for the transplantation protocol was obtained from all patients.

2.2 Transplantation procedure

Patients with malignant hematologic neoplasms received myeloablative or fludarabine-based reduced-intensity conditioning regimens with or without total-body irradiation (TBI) as described elsewhere [27, 28]. Patients with aplastic anemia received conditioning regimens consisting of high-dose cyclophosphamide and horse or rabbit antilymphocyte globulin with or without 2–4 Gy TBI. None of these patients received T-cell-depleted grafts. All patients received GVHD prophylaxis by the use of cyclosporine or tacrolimus combined with or without short-term methotrexate. A proportion of patients given transplants from HLA-mismatched family members or unrelated marrow donors received mycophenolate mofetil in addition to tacrolimus plus methotrexate as GVHD prophylaxis [28]. All patients received supportive care including blood product transfusion and prophylaxis against opportunistic infections according to our institutional protocols [29].

2.3 Evaluation and management of acute and chronic GVHD

All patients were graded for aGVHD using conventional criteria, and the maximum grade until day 100 after transplantation was assigned [30]. Patients who developed grade II–IV aGVHD were initially treated with methylprednisolone or prednisolone usually at a dose of 1–2 mg/kg. Treatment of steroid-refractory aGVHD was variable.

The incidence of cGVHD was retrospectively evaluated by using the NIH consensus criteria [18]. Patients who had at least one "diagnostic" clinical sign or at least one "distinctive" manifestation, confirmed by relevant laboratory tests or histologic examination, were defined as having cGVHD if other possible diagnoses were excluded. Subclassification of cGVHD into "classic cGVHD" and "overlap syndrome" was strictly according to the NIH criteria. If patients had any features of aGVHD along with classic cGVHD, they were classified as having an overlap syndrome. The severity of cGVHD was assessed at its onset and at maximal clinical worsening and graded into "mild", "moderate", and "severe" categories according to the global scoring system defined by the NIH criteria. Treatment of cGVHD was variable, but followed some general principles; patients with isolated mouth, ocular, or localized skin cGVHD were treated only with topical therapy, while patients with more symptomatic cGVHD were treated with systemic immunosuppressive agents such as prednisolone at a dose of 0.5–1.0 mg/kg per day combined with calcineurin inhibitors. Although the duration and dosing of those agents were not standardized, patients typically received treatment until all symptoms of cGVHD were resolved or stabilized. Patients with less severe symptoms were often treated with peroral low-dose prednisolone at a dose of less than 0.5 mg/kg per day.

2.4 Statistical analysis

Descriptive statistics were used to summarize variables related to patient and transplant characteristics. Comparisons among the groups were performed by use of extended Fisher exact test for categorical variables and Wilcoxon–Mann–Whitney test for continuous variables. The primary endpoint of the study was cGSS, which is defined as the time from the day of diagnosis of cGVHD to the day of death in the absence of relapse or secondary malignancy, among patients who developed NIH cGVHD stratified by its subcategory or severity at onset. The probabilities of cGSS were estimated according to the Kaplan–Meier method, and univariable comparison between groups was made using the log-rank test. Patients who were alive without recurrent or secondary malignancy were censored at their last follow-up visit and those who experienced recurrent or secondary malignancy were censored at the time of its diagnosis. The time to discontinuation of IST was defined among patients who received systemic IST for the treatment of NIH cGVHD as the time from the day of diagnosis of cGVHD to the day of withdrawal of systemic IST. NRM was defined among all patients included in the study as rates of death without evidence of primary disease recurrence. The incidence rates of IST withdrawal and those of NRM were estimated with the use of the cumulative incidence method to accommodate the following competing events [31]: the onset of recurrent or secondary malignancy and death from any cause for IST withdrawal, and the recurrent primary disease for NRM. Cox proportional-hazards regression models were used to evaluate variables potentially associated with cGSS, while competing risks regression models were used to evaluate variables potentially associated with IST withdrawal and NRM [32]. The variables included in the analysis were as follows: patient age, donor–recipient sex combination, disease status at the time of transplantation, donor–recipient HLA compatibility, stem cell sources, type of conditioning regimens, grades of prior aGVHD (grades 0–1 vs. grades 2–4), subcategory of NIH cGVHD (classic cGVHD vs. overlap syndrome), global severity of NIH cGVHD at onset (mild to moderate vs. severe), platelet counts, eosinophil counts, and administration of systemic corticosteroids at the onset of cGVHD. In the analysis to evaluate the impact of the presence of each NIH cGVHD subtype on NRM for the entire cohort of patients in the study, development of each subtype of cGVHD was treated as a time-dependent covariate under the assumption that a patient who developed moderate or severe cGVHD could not revert to less severe cGVHD and that classic cGVHD and overlap syndrome could not switch to each other [33]. Factors having two-sided P values less than 0.1 for association with outcome were included in multivariable model using a forward and backward stepwise method with a predetermined risk of 0.1. Two-sided P values <0.05 were considered to be statistically significant. All analyses were performed using STATA version 11 (College Station, TX, USA) according to patient information available as of 1 July 2009.

3 Results

3.1 Patient characteristics

Table 1 shows the characteristics of the 211 patients included in the study; they had a median age of 46 years, included 113 males and 98 females, and underwent transplantation for malignant hematologic neoplasms in most cases. The number of patients who received bone marrow, peripheral blood, and cord blood unit was 152 (72%), 44 (21%), and 15 (7%), respectively. After a median follow-up of 37.2 months (range 3.3–111.6), a total of 96 patients (45%) developed manifestations of cGVHD that met the NIH consensus criteria. There was no statistically significant difference in background characteristics between patients who developed NIH cGVHD and those who did not, except that the former group included higher proportion of patients with a history of antecedent grade II–IV aGVHD.

Table 1 Patient and transplantation characteristics

Characteristic	All patients (n = 211)	NIH cGVHD		
		Absent (n = 115)	Present (n = 96)	P value
Median patient age, years (range)	46 (17–69)	46 (19–69)	47 (17–67)	0.90
Donor/recipient sex combination, n (%)				0.17
Male/male	66 (31)	41 (35)	25 (26)	
Male/female	42 (20)	21 (18)	21 (22)	
Female/female	56 (27)	33 (29)	23 (24)	
Female/male	47 (22)	20 (17)	27 (28)	
Diagnosis, n (%)				0.59
Myeloid neoplasms	113 (54)	65 (57)	48 (50)	
Precursor lymphoid neoplasms	31 (15)	17 (15)	14 (15)	
Mature lymphoid neoplasms	61 (29)	29 (25)	32 (33)	
Aplastic anemia	6 (3)	4 (3)	2 (2)	
Disease status at transplant, n (%)				0.41
Standard risk	105 (50)	54 (47)	51 (53)	
High risk	106 (50)	61 (53)	45 (47)	
Donor type[a], n (%)				0.71
HLA-matched related	83 (39)	45 (39)	38 (40)	
HLA-mismatched related	23 (11)	12 (10)	11 (11)	
HLA-matched unrelated	89 (42)	47 (41)	42 (44)	
HLA-mismatched unrelated	16 (8)	11 (10)	5 (5)	
Donor/recipient HLA compatibility[a], n (%)				0.59
Matched	172 (82)	92 (80)	80 (83)	
Mismatched	39 (18)	23 (20)	16 (17)	
Stem cell source, n (%)				0.30
Bone marrow	152 (72)	85 (74)	67 (70)	
Peripheral blood	44 (21)	20 (17)	24 (25)	
Cord blood	15 (7)	10 (9)	5 (5)	
Conditioning regimen, n (%)				0.55
Myeloablative with TBI	113 (54)	64 (56)	49 (51)	
Myeloablative without TBI	15 (7)	10 (9)	5 (5)	
Reduced intensity with TBI	65 (31)	33 (29)	32 (33)	
Reduced intensity without TBI	18 (9)	8 (7)	10 (10)	
GVHD prophylaxis, n (%)				0.73
Tacrolimus based	169 (80)	91 (79)	78 (81)	
Cyclosporine based	42 (20)	24 (21)	18 (19)	
Prior aGVHD, n (%)				0.048
Grade 0–1	117 (55)	70 (61)	47 (49)	
Grade 2	72 (34)	38 (33)	34 (35)	
Grade 3–4	22 (10)	7 (6)	15 (16)	
Median months (range) after transplantation[b]	37.2 (3.3–111.6)	35.6 (3.3–111.6)	40.6 (4.0–105.3)	0.14

cGVHD chronic graft-versus-host disease, *aGVHD* acute graft-versus-host disease, *TBI* total-body irradiation

[a] HLA matching was defined by 2-digit compatibility at HLA-A, -B, and -DRB1 loci

[b] Median follow-up months among patients who were alive at the time of last follow-up

Table 2 summarizes the characteristics of 96 patients who developed NIH cGVHD according to its subcategory; 77 (80%) developed "classic cGVHD" and 19 (20%) developed "overlap syndrome". A total of 31 (40%) patients with classic GVHD and 18 (95%) with overlap syndrome had a prior history of grade II–IV aGVHD. The

median time from transplantation to the onset of cGVHD in patients with overlap syndrome was shorter compared to patients with classic cGVHD (4.1 vs. 7.1 months, $P < 0.001$). All patients with overlap syndrome were graded as having moderate or severe cGVHD, whereas the proportion of patients who developed severe cGVHD was similar between patients with classic cGVHD and those with overlap syndrome. Proportions of patients with platelet counts less than $100 \times 10^3/\mu L$, eosinophil counts less than $500/\mu L$, and ongoing systemic corticosteroid treatment at the onset of cGVHD were higher among patients who developed overlap syndrome compared with those who developed classic cGVHD.

3.2 Chronic GVHD-specific survival

Of the 96 patients who developed NIH cGVHD, recurrent or secondary malignant neoplasm occurred in 27 patients and death due to any cause occurred in 31 patients. The respective 3-year probabilities of cGSS among patients who developed classic cGVHD and overlap syndrome were 88 and 70% ($P = 0.060$) (Fig. 1a), while those among subgroups of patients graded to have mild, moderate, and severe cGVHD at onset were 100, 86, and 69% (mild to moderate vs. severe, $P = 0.034$) (Fig. 1b). Table 3 shows the results of univariable and multivariable analyses for factors potentially associated with cGSS among the patients who developed NIH cGVHD. In univariable analysis, the presence of severe cGVHD and thrombocytopenia at cGVHD onset were significantly associated with lower cGSS, whereas the presence of an overlap syndrome and high-risk malignant disease tended to be associated with lower cGSS. In multivariable analysis, the presence of thrombocytopenia at cGVHD onset was the only significant factor that adversely affected cGSS [hazard ratio (HR) for mortality = 4.05, 95% confidence interval (CI) = 1.35–12.1, $P = 0.013$], although patients with severe cGVHD (HR = 2.58, 95% CI = 0.90–7.39, $P = 0.077$) or those with high-risk underlying disease (HR = 2.75, 95% CI = 0.86–8.80, $P = 0.088$) also had a trend toward lower cGSS.

3.3 Duration of systemic immunosuppressive treatment

A total of 81 patients received systemic immunosuppressive agents for the treatment of NIH cGVHD. In this group of patients, the cumulative incidence of withdrawal of systemic IST was 40% (95% CI = 29–51%) at 3 years after the onset of cGVHD, while the cumulative incidence of the competing risks of death or recurrent/secondary malignancy during systemic IST was 42% (95% CI = 32–55%) (Fig. 2). In univariable analysis, no significant association was found between discontinuation of IST and subcategory or global severity of NIH cGVHD (overlap syndrome vs. classic cGVHD, HR for IST withdrawal = 0.51, 95% CI = 0.20–1.31, $P = 0.16$; severe vs. mild to moderate, HR = 0.90, 95% CI = 0.42–1.96, $P = 0.80$). Multivariable analysis revealed two factors significantly associated with prolonged administration of systemic IST; high-risk primary disease (HR = 0.39, 95% CI = 0.19–0.77, $P = 0.007$) and the ongoing use of systemic corticosteroids at the onset of cGVHD (HR = 0.40, 95% CI = 0.19–0.84, $P = 0.015$).

3.4 Non-relapse mortality

Death from non-relapse causes occurred in 16 (17%) of 96 patients who developed NIH cGVHD and in 10 (9%) of 115 patients who did not. In a multivariable analysis of the entire series of 211 patients, treating the subcategory or peak severity of NIH cGVHD as a time-dependent covariate, development of the overlap syndrome or severe cGVHD was significantly associated with higher risk of NRM compared to the absence of cGVHD (overlap syndrome vs. no cGVHD, HR = 3.90, 95% CI = 1.32–11.6, $P = 0.014$; severe cGVHD vs. no cGVHD, HR = 6.21, 95% CI = 2.25–17.1, $P < 0.001$). Development of classic cGVHD or mild-to-moderate cGVHD was not significantly associated with higher risk of NRM when compared with the absence of NIH cGVHD (classic cGVHD vs. no cGVHD, HR for mortality = 1.39, 95% CI = 0.55–3.53, $P = 0.49$; mild-to-moderate cGVHD vs. no cGVHD, HR = 2.25, 95% CI = 0.62–8.18, $P = 0.22$).

4 Discussion

In the present study, we evaluated the clinical significance of subcategory and severity of NIH cGVHD in terms of their influences on cGSS, discontinuation of IST, and NRM using a retrospective cohort of patients who underwent allo-HCT for hematologic disorders. In univariable analysis, patients with overlap syndrome tended to have a lower probability of cGSS than those with classic cGVHD, while patients who developed severe cGVHD had significantly worse cGSS compared with those who developed mild-to-moderate cGVHD. Although such differences in cGSS according to NIH cGVHD subtypes did not reach statistical significance by multivariable analysis, patients who developed overlap syndrome or severe NIH cGVHD had a significantly higher NRM than those who did not develop any manifestation of NIH cGVHD. These results suggest that both subcategory and global severity of NIH cGVHD might be useful for evaluating the risk of GVHD-associated mortality in patients diagnosed to have cGVHD by the NIH criteria. In

Table 2 Characteristics of chronic GVHD according to subcategory defined by the National Institutes of Health criteria

Characteristics	Total (n = 96)	NIH cGVHD subcategory		
		Classic cGVHD (n = 77)	Overlap syndrome (n = 19)	P value
Median months (range) to onset of cGVHD	6.7 (2.1–29.9)	7.1 (2.7–29.9)	4.1 (2.1–20.7)	<0.001
Involved organs or sites[a], n (%)[b]				0.92
Skin	55 (57)	40 (52)	15 (79)	
Mouth	69 (72)	56 (73)	13 (68)	
Eyes	29 (30)	23 (30)	6 (32)	
Gastrointestinal tract	34 (35)	25 (32)	9 (47)	
Liver	76 (79)	61 (79)	15 (79)	
Lungs	12 (12)	9 (12)	3 (16)	
Joints and fascia	4 (4)	3 (4)	1 (5)	
Genital tract	2 (2)	2 (3)	0 (0)	
Number of involved organs or sites[a], n (%)				0.14
1	7 (7)	7 (9)	0 (0)	
2	27 (28)	24 (31)	3 (16)	
3 or more	62 (65)	46 (60)	16 (84)	
Maximum score of involved organs[a], n (%)				0.18
Score 1	22 (23)	20 (26)	2 (11)	
Score 2 (other than lungs)	26 (27)	18 (62)	8 (42)	
Score 2 (lungs)	6 (6)	4 (5)	2 (11)	
Score 3	42 (44)	35 (45)	7 (37)	
Severity at onset, n (%)				0.023
Mild	20 (21)	20 (26)	0 (0)	
Moderate	53 (55)	39 (51)	14 (74)	
Severe	23 (24)	18 (23)	5 (26)	
Severity at peak, n (%)				0.17
Mild	12 (13)	12 (16)	0 (0)	
Moderate	39 (41)	29 (38)	10 (53)	
Severe	45 (47)	36 (47)	9 (47)	
Platelet count at cGVHD onset, n (%)				0.002
$100 \times 10^3/\mu L$ or more	65 (68)	58 (75)	7 (37)	
Less than $100 \times 10^3/\mu L$	31 (32)	19 (25)	12 (63)	
Eosinophil count at cGVHD onset, n (%)				0.010
Less than 500/μL	68 (71)	50 (65)	18 (95)	
500/μL or more	28 (29)	27 (35)	1 (5)	
Systemic corticosteroids at cGVHD onset, n (%)				<0.001
Not received	63 (66)	61 (79)	2 (11)	
Received	33 (34)	16 (21)	17 (89)	

cGVHD chronic graft-versus-host disease

[a] Data evaluated at peak clinical worsening are shown

[b] The sum of the number per involved site is not equal to the number of evaluable patients, because the involvement of more than one organ can occur in a single patient. Accordingly, the sum of percentage among the total number of patients does not equal to one hundred

contrast, duration of IST was neither affected by NIH cGVHD subcategory nor by its severity.

While cGSS has been frequently used as a study endpoint to describe the mortality attributable to cGVHD-associated organ dysfunction, there have been no established early surrogates that help to guide the clinical management of patients with evidence of ongoing cGVHD. Given that the historic limited/extensive grading system is not a useful predictor for the severity of organ involvement in terms of mortality risk, several studies have attempted to develop