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G. 知的財産権の出願・登録状況
該当なし

Ⅱ．研究成果の刊行に関する一覧表

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Ⅲ. 研究成果の刊行物

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Minimal Residual Disease following Allogeneic Hematopoietic Stem Cell Transplantation

Nicolaus Kröger,[1] Koichi Miyamura,[2] Michael R. Bishop[3]

Minimal residual disease (MRD), both before and after transplantation, is a clinically important yet relatively poorly defined aspect of allogeneic hematopoietic stem cell transplantation (alloHSCT). The clinical relevance of MRD in the context of alloHSCT has been demonstrated by its association with the development of clinical relapse. However, with the possible exception of chronic myeloid leukemia (CML), the specific techniques, timing, frequency, and clinical utility, relative to improvement in patient outcomes, for monitoring MRD in the setting of alloHSCT has yet to be clearly defined. A concise overview of monitoring techniques for detecting MRD, as well as treatment strategies and biological and clinical research initiatives for MRD suggested by the National Cancer Institute First International Workshop on the Biology, Prevention, and Treatment of Relapse after Allogeneic Hematopoietic Stem Cell Transplantation, is covered in this article.

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KEY WORDS: Minimal residual disease, Allogeneic, Relapse, Graft-versus-tumor, DLI

INTRODUCTION

Minimal residual disease (MRD), in the setting of allogeneic hematopoietic stem cell transplantation (alloHSCT), poses several interesting questions and complex challenges. The relevance of these questions and challenges is personified by the relationship between MRD and the risk of relapse, which is primary cause of treatment failure and death after alloHSCT [1]. The clinical relation of posttransplant MRD with relapse, particularly in relationship to chronic myeloid leukemia (CML), was recognized early with development of cytogenetic and molecular techniques of detection [2]. The clinical relevance of MRD has been further recognized with the increased use of nonmyeloablative and reduced-intensity conditioning (RIC) regimens, with which relapse is even a greater clinical problem [3,4].

Despite the clear association of MRD with relapse, the clinical relevance of MRD in the alloHSCT setting remains to be determined. First and foremost, the definition of MRD needs to be defined for each disease, and needs to be distinguished from what we currently refer to as "remission" or "relapse." The detection of persistent disease posttransplant by immunophenotypic measures has significantly different implications for patients with acute lymphocytic leukemia (ALL) compared to someone with persistent chronic lymphocytic leukemia (CLL) [5,6]. Similarly, the molecular detection of a cytogenetic abnormality in the posttransplant is markedly different for a patient transplanted with chronic myeloid leukemia (CML) compared to a patient with acute myeloid leukemia (AML) [7]. Second, when and how often we should be using available techniques for a specific disease remains to be defined. This applies not only to the posttransplant setting, but also to the pretransplant setting, where multiple studies have demonstrated the prognostic significance of MRD prior to conditioning [8]. As the majority of relapses occur within the first 6 months after transplantation [1], it is important to determine the frequency of monitoring for recurrent disease within this posttransplant period. If we can determine when and how often, the next question is what tests should we be performing and are those tests adequately sensitive, specific, reproducible, practical, and economical. Finally, and most importantly, does monitoring for MRD make a clinical difference? There is sufficient evidence that detection of MRD provides prognostic information. However, does this information result in clinical decisions, relative to choice of conditioning regimen or stem cell product relative to detection of pretransplant MRD or intervention (eg, withdrawal of immune suppression

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or donor lymphocyte infusion) that result in improved outcomes? These remain essential questions for which there are relatively limited data and recommendations, with the possible exceptions of CML and ALL, and even with these diseases, there remains a need for further investigation.

This manuscript attempts to provide a concise overview of many of these issues. Specifically, it attempts to address methods for monitoring MRD and strategies to clinically manage patients once MRD is detected. In addition, a brief summary is provided on the National Cancer Institute First International Workshop on the Biology, Prevention, and Treatment of Relapse after Allogeneic Hematopoietic Stem Cell Transplantation, which attempted to address in a formal manner many of the issues described above.

MONITORING MRD AFTER ALLOSCT

Improved supportive care, the introduction of RIC regimen, and careful donor selection have substantially decreased the nonrelapse mortality (NRM) after alloHSCT in recent years, and therefore relapse has become the leading cause of death following alloHSCT. Furthermore, as inferred above, relapse remains the primary cause of death among patients surviving more than 2 years after alloHSCT [9]. Despite improved understanding of the biology that underlies the graft-versus-leukemia/tumor (GVL/GVT) effect, the relapse rate has not decreased over the past 20 years [10,11]. It is obvious that relapse after alloHSCT evolves from residual disease that escaped the preceding conditioning regimen as well as the graft-versus-malignancy effect.

New methodologic and technologic advances allow sensitive detection of MRD and early recognition of recurrence after alloHSCT. This is of clinical importance because intervention prior to florid relapse improves outcome for certain hematologic malignancies [12,13]. Standard diagnostic criteria that are widely employed in the definition of relapse for the different hematologic malignancies are based on morphologic bone marrow investigations, imaging, and/or specific laboratory findings. After alloHSCT, more sensitive methods, such as tumor-specific molecular primers, molecular genetics, fluorescence in situ hybridization (FISH), flow cytometry, and/or chimerism analysis, are commonly used to monitor patients with respect to relapse (Table 1).

Broadly, 2 different approaches are mainly used for the posttransplant surveillance of disease status: characterization of chimerism, and specific detection of MRD. The latter approach measures the malignant clone directly, whereas chimerism assessment characterizes the origin of posttransplant hematopoiesis. For chimerism as well as for specific detection of residual disease, a variety of techniques are available, although in general, there have been more studies looking directly at markers of residual tumor than of chimerism [14]. Despite the increasing sensitivity by the described methods of chimerism determination, because of its low specificity, this method is not a reliable means of detecting MRD. The specificity is higher in diseases that originate from a stem or progenitor cell (eg, AML, CML), whereas in B cell lymphoma or multiple myeloma, which originate from a late B cell stage of development, the specificity of chimerism to detect MRD or relapse is low. The lack of specificity might be overcome partly by performing lineage-specific chimerism in some diseases such as multiple myeloma [15].

A paradigm for the importance of minimal molecular disease and prediction of relapse after alloHSCT is CML. Here, it is now well established that the detection of the chimeric *BCR-ABL* mRNA transcript by reverse-transcriptase polymerase chain reaction (RT-PCR) is a powerful predictor of subsequent relapse [16]. The use of quantitative PCR has greatly increased the clinical value of monitoring MRD. It could be demonstrated that the kinetics of BCR-ABL level over time described impending relapse and response to donor lymphocyte infusion (DLI). Low or absence of residual BCR-ABL was associated with a very low risk of relapse (1%), compared to 75% relapse rate in CML patients with increasing or persistently high BCR-ABL levels [17]. The activating mutation V617F of the *JAK2* gene is an obvious target for monitoring MRD in patients with myeloproliferative disorders undergoing alloHSCT. There are emerging data suggesting that, similar to *BCR-ABL* in CML, PCR negativity for *JAK2-V617F* correlates with prolonged remission and that reappearance of a detectable *JAK2-V617F* clone is associated with relapse [18].

However, the utility of the available tools in the monitoring of disease status after alloHSCT has not yet been fully elucidated across all hematologic malignancies. In AML and myelodysplastic syndromes, several studies demonstrated the relevance of chimerism, and especially its kinetics, for the prediction of relapse. A variety of genetic markers are available for MRD in AML such as rearrangements t (15;17)/*PML-RARA*, inv(16)/*CBFB-MYH11*, and t(8;21)/*RUNX1-RUNX1T1*, *NPM1*, *FLT3*, or *MLL-PTD* but have not been studied in a larger cohort of patients.

Methods for MRD monitoring in B- or T-lymphoid malignancies include PCR techniques aiming to quantitatively detect disease specific T cell receptor (TCR) or immunoglobulin (Ig) gene rearrangements. Multiple studies support the independent prognostic value of MRD measurements in pediatric and adult patients with B- and T-lineage ALL. Furthermore, the risk of relapse appears to be proportional to the level of MRD, which in some studies was found to be the most powerful prognostic factor for relapse in

Table 1. Diagnostic Methods to Monitor Residual Disease and Relapse after Allogeneic Stem Cell Transplantation

Detection of Residual Disease (MRD)	Karyotyping	FISH	Flow Cytometry	Antigen Receptor PCR	Translocation or Other mRNA PCR	Chimerisms: XY FISH	Chimerism: qPCR/VNTR-PCR
Utility	Subset of all types of neoplasms with chromosomal abnormalities	Subset of all types of neoplasms with know chromosomal abnormality	ALL; most AML; CLL; myeloma	ALL; lymphoma, myeloma	CML; subset of ALL; subset of AML; subset of lymphoma	All types of neoplasms (sex mismatched SCT) Disadvantage not specific for MRD	All neoplasms (precondition differences in donor/ recipient polymorphisms) Disadvantage: not specific for MRD
Sensitivity	10^{-1}	10^{-2}	10^{-3}-10^{-4}	10^{-4}-10^{-5}	10^{-3}-10^{-6}	10^{-2}	10^{-3}-10^{-6}

qPCR indicates quantitative real-time PCR (modified after [6]); CML, chronic myeloid leukemia; ALL, acute lymphoblastic leukemia; AML, acute myelogenous leukemia; VNTR, variable number tandem repeat.

multivariate analyses [13]. Similarly, detection of pretransplant MRD in pediatric and some adult studies is highly predictive of relapse following alloHSCT and, coupled with posttransplant MRD evaluation, may guide early posttransplant intervention such as early withdrawal of immunosuppression, administration of DLI, or addition of posttransplant maintenance therapy (eg, targeted tyrosine kinase inhibition for Ph+ ALL).

In CLL, 2 main approaches of MRD assessment have been followed: flow cytometry, taking advantage of the unique immunophenotype of CLL, and PCR-based strategies using the clonal rearrangement of the hypervariable region of the V_H part of the immunoglobulin heavy chain gene (CDR3 region). Several studies showed that MRD assessment after alloHSCT is predictive for durable freedom from CLL progression if: (1) MRD levels are below 1×10^{-4} at 1 year posttransplant, or (2) show decreasing or stable kinetics within the quantitative range. The clinical impact of MRD detection in different lymphomas is not identical.

Specific chromosomal translocations detectable by PCR amplification, particularly t(11;14) and t(14;18) translocation, are present in mantle cell lymphoma and follicular lymphoma, respectively, but t(14;18) translocation is also detectable by PCR at low levels in 10% to 25% of healthy individuals. For Hodgkin lymphoma, neither cytogenetics, flow cytometry, nor molecular testing is helpful for assessing residual disease [19].

In multiple myeloma, MRD can be detected by PCR using patient-specific primers derived from the rearrangement of immunoglobulin heavy-chain genes. It could be shown that durable PCR-negativity after allografting had a cumulative risk of relapse at 5 years of 0%, in comparison to 33% for PCR-mixed patients and 100% for patients who never achieved PCR-negativity [20]

Ongoing and further clinical trials investigate whether sensitive MRD detection will allow for earlier therapeutic intervention, and it is hoped that treatment prior to overt relapse may improve outcome of allogeneic stem cell transplantation for hematologic malignancies.

STRATEGIES AND OPTIONS FOR RECURRENT DISEASE FOLLOWING ALLOSCT

The clinical significance of MRD after alloHSCT is different among diseases. MRD has been extensively studied using the qualitative PCR method during the early 1990s. Detection of BCR-ABL by PCR in the first year after alloHSCT for CML patients disappears in the majority of patients, secondary to ongoing GVT

effects; however, detection of MRD after alloHSCT for Philadelphia chromosome-positive acute lymphoblastic leukemia (Ph+ALL) is indicative of imminent hematologic relapse [21-24]. In the case with t(8;21) AML, MRD after chemotherapy does not always indicate eventual clinical relapse. In the last decade, quantitative PCR machines are widely available, and sequential and quantitative tests of leukemic genes have become available. With this technique, a rise in the amount of leukemic genes strongly suggests clinical relapse in the near future. Also, several investigators have tried to find thresholds for the amount of genes that are predictive of clinical relapse. However, because of a lack of standardization of this technique, hitherto universal threshold has not been clarified at any leukemia with the possible exception of CML.

Clinical Intervention

Because of the limitation of quantitative PCR as mentioned above, clinical intervention upon the emergence of MRD has not been well established. Clinical interventions for early relapse and MRD after alloHSCT are performed in 2 ways; 1 is adoptive immunotherapy including DLI and vaccination, and the other is administration of new agents, which are expected to preserve normal hematopoietic cells. Several questions are raised in this clinical setting. First, does early intervention have more clinical effects than the intervention performed at hematological relapse? Second, does clinical intervention affect the other parameters such as graft-versus-host disease (GVHD), related adverse events, and the subsequent alloHSCT. Third, which is the better way, prophylactic administration or intervention upon MRD, for patients with a high risk of relapse?

Adoptive Immunotherapy

DLI was first developed for relapsed patients. Although they are dramatically effective for CML, DLI remains limited of limited utility for patients with other diseases because of inadequate responses and toxicity related to GVHD, which occurs in one-third of patients. As a strategy to reduce the incidence and severity of GVHD while preserving the GVL effect, tumor-specific DLI are proposed [25]. A protocol to generate hematopoietic cell–specific minor antigen (eg, HA-1, HA-2, ACC-1) specific T cell lines from mHag-negative donors was studied for adoptive immunotherapy. Warren et al. [26] conducted a phase I/II study to test the toxicity and effectiveness of CTL clones specific for minor H antigens. However, this strategy using cloned antigen-specific T cells has been shown to be ineffective mostly because these cells could not survive long enough to execute their cytotoxic ability in vivo. This problem could be overcome by: (1) infusion of a relatively young and small number of memory T cells without extensive expansion in vitro, and (2) infusion of autologous peripheral blood T cells transduced retrovirally with T cell receptor α and β cDNA cloned from tumor/minor antigen–specific T cell clones [27]. The latter approach has been shown to be promising in the setting of melanoma treatment in studies conducted by Rosenberg and colleagues at the National Cancer Institute [28]. Thus, T cells armed with TCR specific for WT-1, HA-1, HA-2, and ACC-1 would be great candidates for adoptive immunotherapy in the very near future. Another approach studied intensively in the clinical hematology field is a vaccination using epitope peptides such as WT-1, PR3, MUC-1, NY-ESO-1, and BCR-ABL fusion polypeptides. In particular, WT-1 is one of the most promising tumor antigens because WT1 vaccination-driven immunologic responses and clinical responses, including reduction of leukemic cells, and the reduction of the M-protein amount in myeloma, have been reported. Further enhancement of the efficacy of the WT1 peptide vaccine can be expected by coadministration of WT1-specific helper peptide, Th1-inducing adjuvant, or immunosuppressive chemotherapy prior to vaccinations to take advantage of inhibition of regulatory T cells and facilitation of homeostatic expansion of desired T cells. Adoptive immune therapies as prophylaxis or preemptive therapy would be performed in the near future.

New Agents

Chemotherapy for the patients with recurrent disease is hampered by the fact that these agents impel the normal hematopoietic cells, as well as the fact that tumor cells and tumor-specific agents have long been desired. Recently, a new molecular-specific targeting agent has been developed. The specific manner of these new agents prompts us to use them for earlier interventions. Nevertheless, most of these tumor-specific agents exert some effects on normal hematopoietic cells and interfere with immunologic functions after alloHSCT.

Tyrosine kinase inhibitors

Philadelphia chromosome-positive ALL is associated with highly aggressive disease. Although alloHSCT is at present the only curative treatment option, hematologic relapse still remains a major obstacle. Recently, there have been some reports of posttransplant imatinib administration, but its efficacy and administration methods are still controversial. Nishiwaki and colleagues [29] compared prophylactic administration of imatinib with intervention upon molecular relapse to evaluate the effect of posttransplant imatinib administration. MRD became positive in both groups, leading to hematologic relapse. It was therefore concluded that

posttransplant imatinib administration may not be an ideal prophylactic treatment for Ph+ALL patients. In contrast, Ottmann et al. [30] demonstrated that all Ph+ALL patients who received imatinib upon appearance of BCR-ABL and promptly achieved molecular response remained in remission for the duration of imatinib treatment.

Bortezomib

Recently, both conventional chemotherapy and autologous and alloHSCT combined with new agents, such as thalidomide, lenalidomide, and bortezomib, have improved the depth of response and survival of multiple myeloma patients. However, after transplantation, most patients still harbor residual disease. Ladetto et al. [31] reported the effect of posttransplant consolidation including bortezomib on MRD detected by PCR using tumor-clone–specific primers. Molecular remissions were achieved in 3% of patients after autologous HSCT and 18% after consolidation with bortezomib. It has been proposed that bortezomib increases the expression of Fas and DR5 and enhances GVT effects, and that this agent also suppresses the activity of NFkB, resulting in reduction of inflammatory cytokines related to graft-versus-host activity [32].

Lenalidomide

Lenalidomide is an immunomodulatory drug (IMiD) that has multiple effects on myeloma cells and their microenvironment. Administration of IMiDs for postautologous HSCT maintenance resulted in prolonged progression-free survival (PFS) even in patients who achieved very good partial response or complete response before lenalidomide administration. In the alloHSCT setting, lenalidomide plus low-dose dexamethasone combination therapy have shown significant disease and chronic GVHD (cGHVD) control for myeloma patients, who relapsed after transplantation [33]. GVHD control with IMiD is still controversial but a very attractive issue for investigation [34].

Hypomethylating agents

Low-dose 5-azacitidine (5-Aza) was used by investigators at the M.D. Anderson Cancer Center for patients with AML/MDS as a maintenance therapy or salvage therapy upon relapse after alloHSCT; an overall survival rate of 90% at 1 year was reported [35]. Additive effects of DLI to 5-Aza were also reported. The administration of 5-Aza was not associated with an increased incidence of GVHD. Sanchez-Abarca et al. [36] reported that 5-Aza inhibits T cell proliferation and activation, blocking the cell cycle in the G0 to G1 phase and decreasing the production of proinflammatory cytokines such as tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ). They also reported that administration of 5-Aza after transplantation prevented the development of GVHD, leading to a significant increase in survival in a fully mismatched bone marrow transplantation mouse model. Recently, decitabine, another DNA hypomethylating agent, was reported to be used in patients experiencing cytogenetic relapse after alloHSCT [37].

Humanized monoclonal antibodies

Rituximab (anti-CD20 monoclonal antibody) was used for 9 chronic lymphocytic leukemia patients who had persistent disease after alloHSCT and underwent immuno-manipulation to augment GVT effects including immunosuppression withdrawal and DLI with rituximab treatment, and 8 patients had a complete response [38]. Alemtuzumab (anti-CD52 monoclonal antibody), as well as antithymocyte globulin (ATG), has been used as a T cell depletion method in alloHSCT. Because it is reported that the majority of precursor B-ALL blasts express CD52, and CD52 is expressed on other ALL cells, alemtuzumab is considered to potentially contribute to the eradication of MRD [39].

Summary on the Treatment of MRD

For decades, interventions for relapsed patients have been performed using DLI and chemotherapies; however, they are a 2-edged sword, hampering normal hematopoietic cells as well as tumor cells. Recently, the emergence of new strategies using tumor-specific DLI and tumor-specific new agents has prompted us to use these methods before clinical relapse. Some of them are used as prophylaxis, and some of them are used upon tumor emergence at molecular level. Trials confirming these strategies are just beginning, and there is a need for the definition of MRD. Thus, it is becoming more and more important that the measurement of MRD becomes standard practice; otherwise, clinical studies will be somewhat meaningless.

NATIONAL CANCER INSTITUTE FIRST INTERNATIONAL WORKSHOP ON THE BIOLOGY, PREVENTION, AND TREATMENT OF RELAPSE AFTER ALLOHSCT

As stated above, there is a strong association of MRD with relapse following alloHSCT. The growing recognition of relapse as one of the most significant posttransplant problems led to the organization and convening of the National Cancer Institute First International Workshop on the Biology, Prevention, and Treatment of Relapse after AlloHSCT [40]. The primary objectives of the Workshop were to review the current "state-of-the-science" relative to the biology, natural history, prevention, and treatment, and identify the most important biological and clinical questions that need to be addressed relative to relapse following alloHSCT.

The Workshop, which took place on November 2 and 3, 2009, in Bethesda, Maryland, USA, brought together an international group of more than 200 basic and clinical researchers. Over 50 formal presentations were made by the Workshop committee members that addressed both GVT and non-GVT biology, relapse epidemiology, and natural history, strategies, and therapies for prevention, disease-specific methods, and strategies for monitoring, and disease-specific treatment of relapse following alloHSCT. These presentations are available for viewing at https://ccrod.cancer.gov/confluence/display/NCIRelapse/Presentations+from+Workshop. Each of the 6 workshop committees subsequently prepared a "state-of-the-science" manuscript, which contained their commended research priorities; these manuscripts were published sequentially during 2010 in the *Biology of Blood and Marrow Transplantation* [1,14,19,41-44].

The central Workshop theme was that in its most simplistic form, relapse occurs because tumor cells are first able to resist the cytotoxic effects of the conditioning regimen. These surviving cells either never respond to initial GVT or they subsequently escape from GVT effects after initial control.

Central and recurrent research themes included the necessity to establish biorepositories to collect and store tumor samples before transplant when possible, and after transplant, store samples from allografts for analysis, and collect blood and serum samples at set posttransplant time points and at the time of relapse for study of immunology related to relapse. Second, there is a need for more careful study of the natural history of relapse for specific diseases, particularly in regard to MRD. To perform such studies, there needs to be international acceptance of standard definitions and techniques; it is hoped that the definitions and techniques proposed by the Workshop will be considered for this purpose. Finally, there needs to be multiinstitutional collaboration in regard to prevention and treatment of relapse after alloHSCT. A formal summary of the workshop recommendations will be presented during the 2011 Tandem Transplant Meetings Educational Sessions.

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ORIGINAL ARTICLE

Unrelated cord blood transplantation vs related transplantation with HLA 1-antigen mismatch in the graft-versus-host direction

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Little information is available regarding whether an unrelated cord blood (UCB) unit or a related donor with a 1-antigen mismatch at the HLA-A, HLA-B or HLA-DR locus in the graft-versus-host direction (RD/1AG-MM-GVH) should be selected as an alternative donor for patients without an HLA-matched related/unrelated donor. Therefore, we conducted a retrospective study using national registry data on patients with leukemia or myelodysplastic syndrome who received transplantation using a single UCB ($n = 2288$) unit or an RD/1AG-MM-GVH ($n = 525$). We found that the survival rate in the UCB group was comparable to that in the RD/1AG-MM-GVH group, although the RD/1AG-MM-GVH group with an HLA-B mismatch showed significantly higher overall and non-relapse mortality. Neutrophil and platelet engraftment were significantly faster, whereas the incidence of acute or chronic graft-versus-host disease (GVHD) was significantly higher in the RD/1AG-MM-GVH group. The incidence of acute or chronic GVHD in the RD/1AG-MM-GVH group with *in vivo* T-cell depletion was comparable to that in the UCB group, which translated into a trend toward better overall survival, regardless of the presence of an HLA-B mismatch. In conclusion, UCB and RD/1AG-MM-GVH are comparable for use as an alternative donor, except for RD/1AG-MM-GVH involving an HLA-B mismatch.

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INTRODUCTION

For patients who lack an HLA-identical sibling, an HLA-matched unrelated donor (MUD) is considered to be the preferred alternative donor in allogeneic hematopoietic cell transplantation (HCT).[1–5] However, it is difficult to find an MUD for patients with rare HLA haplotypes. Furthermore, it takes at least a few months from the start of an unrelated donor search to actually receive a graft. Therefore, there is a large demand for an alternative source to an HLA-identical sibling or MUD, particularly for patients who have a rare haplotype or who need immediate transplantation.

Unrelated cord blood (UCB) has emerged as a promising alternative source for pediatric and adult patients.[6–17] In UCB transplantation, up to two antigen/allele mismatches between a recipient and cord blood unit are acceptable without an increased risk of acute graft-versus-host disease (GVHD). The clinical outcome in UCB transplantation is improving, and is almost comparable to that in HLA 8/8 allele MUD transplantation, although a high risk of graft failure and early treatment-related complications are still major issues.[15–17]

Another alternative source is an HLA-mismatched related donor, particularly when a related donor with a 1-antigen mismatch at the HLA-A, HLA-B, or HLA-DR locus in the graft-versus-host (GVH) direction (RD/1AG-MM-GVH) is available. HCT from an RD/1AG-MM-GVH results in a higher but acceptable incidence of acute GVHD.[18–20] In previous studies, HLA mismatches in the host-versus-graft (HVG) direction were associated with a higher incidence of graft failure and lower overall survival (OS).[18,19,21] However, the risk of graft failure might have been improved by the use of conditioning regimens that strongly suppress the recipient's immune system.[22] Therefore, in current clinical practice in Japan, stem cell transplantation from an RD/1AG-MM-GVH is being performed while accepting multiple antigen mismatches in the HVG direction without specific *ex vivo* stem cell manipulation.[18,19,23] We have recently reported that OS in transplantation from an RD/1AG-MM-GVH involving an HLA-B antigen mismatch was inferior, whereas that from an RD/1AG-MM-GVH involving an HLA-A or -DR antigen mismatch was comparable to that from an 8/8-MUD in standard-risk diseases.[23]

Unlike transplantation from an MUD, transplantation using a UCB unit or an RD/1AG-MM-GVH can be performed immediately when necessary. However, little information is available regarding the priority in selecting these alternative donors. Therefore, we conducted a retrospective study using national registry data on 2813 patients with leukemia or myelodysplastic syndrome (MDS)

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who received transplantation using a single UCB or an RD/1AG-MM-GVH.

MATERIALS AND METHODS

Data collection

Data for patients (age: ⩾16 years) with acute myeloid leukemia, acute lymphoblastic leukemia, MDS and chronic myelogenous leukemia who received a first HCT using a single HLA 0–2 antigen-mismatched UCB unit or an RD/1AG-MM-GVH between 1 January 1998 and 31 December 2009 were obtained from the Transplant Registry Unified Management Program (TRUMP),[24] which includes data from the Japan Cord Blood Bank Network (JCBBN) and the Japan Society for Hematopoietic Cell Transplantation (JSHCT). Our analysis included 2306 patients who received a single UCB graft (UCB group) and 541 patients who received a graft from an RD/1AG-MM-GVH (RD/1AG-MM-GVH group). As of January 2012, double UCB grafts for HCT are not available in Japan. The following patients were excluded: 26 patients who lacked data on survival status, survival date, sex of recipient, or GVHD prophylaxis and 8 patients who received stem cells that had been manipulated by *ex vivo* T-cell depletion or CD34 selection. Overall, 2288 patients who received a UCB unit and 525 who received a graft from an RD/1AG-MM-GVH fulfilled the criteria. The study was approved by the data management committees of TRUMP and by the institutional review boards of Japanese Red Cross Nagoya First Hospital and Saitama Medical Center, Jichi Medical University, where this study was organized.

Histocompatibility

Histocompatibility data for the HLA-A, HLA-B and HLA-DR loci were obtained from reports from the institution where the transplantation was performed or from cord blood banks. To reflect current practice in Japan, HLA matching in UCB or RD/1AG-MM-GVH transplantation was assessed by serological data for HLA-A, HLA-B, and HLA-DR loci. An HLA mismatch in the GVH direction was defined as when the recipient's antigens or alleles were not shared by the donor, whereas a mismatch in the HVG direction was defined as when the donor's antigens or alleles were not shared by the recipient.

End points

The primary end point of the study was to compare OS rates between the UCB and RD/1AG-MM-GVH groups. Other end points were the cumulative incidences of neutrophil and platelet engraftment, acute and chronic GVHD, relapse, and non-relapse mortality (NRM). Neutrophil recovery was considered to have occurred when the absolute neutrophil count exceeded 0.5×10^9/l for 3 consecutive days following transplantation. Platelet recovery was considered to have occurred when the absolute platelet count exceeded 50×10^9/l without platelet transfusion. The physicians who performed transplantation at each center diagnosed and graded acute and chronic GVHD according to the traditional criteria.[25,26] The incidence of chronic GVHD was evaluated in patients who survived for at least 100 days.

Statistical analysis

Descriptive statistics were used to summarize variables related to the patient characteristics. Comparisons between groups were performed with the χ^2-test or extended Fisher's exact test as appropriate for categorical variables and the Mann–Whitney *U*-test for continuous variables. The probability of OS was estimated according to the Kaplan–Meier method, and the groups were compared with the log-rank test. The adjusted probability of OS was estimated according to the Cox proportional-hazards model, with other significant variables considered in the final multivariate model. The probabilities of neutrophil and platelet engraftment, acute and chronic GVHD, NRM, and relapse were estimated on the basis of cumulative incidence methods, and the groups were compared with the Gray test;[27,28] competing events were death without engraftment for neutrophil and platelet engraftment, death or relapse without GVHD for acute and chronic GVHD, death without relapse for relapse, and relapse for NRM. The Cox proportional-hazards model was used to evaluate variables that may affect OS, whereas the Fine and Gray proportional-hazards model was used to evaluate variables that may affect engraftment, GVHD, NRM and relapse.[29] We classified the conditioning regimen as myeloablative if either total body irradiation >8 Gy, oral busulfan ⩾9 mg/kg, intravenous busulfan ⩾7.2 mg/kg, or melphalan >140 mg/m^2 was used in the conditioning regimen, and otherwise classified it as reduced intensity, based on the report by the Center for International Blood and Marrow Transplant Research.[30] For patients for whom the doses of agents used in the conditioning regimen were not available, we used the information on conditioning intensity (myeloablative or reduced intensity) reported by the treating clinicians. Acute leukemia in the first or second remission, chronic myelogenous leukemia in the first or second chronic phase or accelerated phase, and MDS with refractory anemia or refractory anemia with ringed sideroblasts were defined as standard-risk diseases, and other conditions were defined as high-risk diseases. The following variables were considered when comparing the UCB and RD/1AG-MM-GVH groups: the recipient's age group (⩽50 years or >50 years at transplantation), sex of recipient, disease (acute myeloid leukemia, acute lymphoblastic leukemia, chronic myelogenous leukemia or MDS), disease status before transplantation (standard- or high-risk), type of conditioning regimen (myeloablative or reduced intensity), type of GVHD prophylaxis (calcineurin inhibitor and methotrexate, calcineurin inhibitor only, or other), year of transplantation (1998–2004, 2005–2009), and the time from diagnosis to transplantation (<6 months or ⩾6 months). In the analysis within the RD/1AG-MM-GVH group, the use of *in vivo* T cell depletion (no vs yes), stem cell source (peripheral blood (PB) stem cells vs bone marrow (BM)), and the number of HLA mismatches in the HVG direction (0–1 vs 2–3) were also considered. Factors without a variable of main interest were selected in a stepwise manner from the model with a variable retention criterion of $P<0.05$. We then added a variable of main interest to the final model. All tests were two-sided, and $P<0.05$ was considered to indicate statistical significance. All statistical analyses were performed with Stata version 12 (Stata Corp., College Station, TX, USA) and EZR (Saitama Medical Center, Jichi Medical University, Saitama, Japan).[31] EZR is a graphical user interface for R (The R Foundation for Statistical Computing, version 2.13.0, Vienna, Austria). More precisely, it is a modified version of R commander (version 1.6–3) that was designed to add statistical functions that are frequently used in biostatistics.

RESULTS

Characteristics of patients and transplants

Table 1 shows the patient and transplant characteristics. Recipients of an RD/1AG-MM-GVH were younger than recipients of a UCB unit. Approximately half of the recipients in the RD/1AG-MM-GVH group received PB. The number of HLA mismatches in the GVH direction between a UCB unit and recipient was 0 in 10%, 1 in 33% and 2 in 57%. In the RD/1AG-MM-GVH group, the number of antigen mismatches in the HVG direction was 0 in 12%, 1 in 68%, 2 in 18% and 3 in 3%. Most of the recipients of an RD/1AG-MM-GVH received a calcineurin inhibitor with methotrexate for GVHD prophylaxis, whereas 25% of UCB recipients received only calcineurin inhibitor. *In vivo* T-cell depletion including antithymocyte globulin (ATG) or alemtuzumab was used in 10% of the RD/1AG-MM-GVH group, but in only 1% of the UCB group. Alemtuzumab was used in only one patient, who received transplantation from an RD/1AG-MM-GVH. Information regarding the dose and type of ATG was missing in two-third of the patients who received ATG. Available data showed that the median dose of thymoglobulin was 2.5 (range 2.5–9.0, $n=9$) and 2.5 (range 1.25–5.0, $n=10$) mg/kg and the median dose of ATG-Fresenius was 8.0 (range 5.0–10.0, $n=3$) and 8.0 (range 5.0–10.0, $n=7$) mg/kg, in the UCB and RD/1AG-MM-GVH groups, respectively. Two-third of UCB transplantations were performed between 2005 and 2009. The median duration of follow-up for survivors was 2 and 4 years in the UCB and RD/1AG-MM-GVH groups, respectively.

Neutrophil and platelet engraftment

The incidence of neutrophil engraftment at day 50 in the RD/1AG-MM-GVH group was higher than that in the UCB group (UCB group, 73%, 95% confidence interval (CI), 71–75%; RD/1AG-MM-GVH group, 93%, 95% CI, 91–95%; Gray test, $P<0.001$; Figure 1a). The incidence of platelet engraftment at day 150 in the