Blood and Marrow TRANSPLANTATION

REVIEWS

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IN THIS ISSUE

New Entries to the Recipe Book of Allogeneic Transplantation

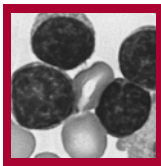
by John R. Wingard, MD, Editor

The three prime ingredients of allogeneic hematopoietic cell transplantation (HCT) are conditioning regimen, graft, and posttransplantation supportive care. For decades, much of the research interest has focused on the latter two ingredients. Characterization of graft hematopoietic and immune constituents and manipulation of these elements have led to important insights, new knowledge, and improved outcomes. Similarly, development and testing of hematopoietic growth factors, new immunosuppressive regimens, and new antimicrobial agents have resulted in improved outcomes. Meantime, interest in conditioning regimens languished. The backbones of most regimens have remained some mix of total body irradiation, busulfan, or cyclophosphamide.

In recent years all that has changed. Indeed, testing of new conditioning regimens has become hot again and new recipes are being sampled. Much of this renewed interest has come about because of fundamental changes in our concepts of how allogeneic HCT cures cancer: less about the brute force of intensive cytotoxic bludgeoning of cancer cells, and more about facilitating immunotherapy. Much of this new emphasis has been made possible by new immunologic agents such as the purine analogues, potent T-cell antibody preparations, and more recently new ways to deliver irradiation by radioimmunoconjugates or lymphoid instead of total body irradiation.

This issue contains a transcript of a symposium, which was presented at the 2005 BMT Tandem Meetings in Keystone, Colorado, that addresses the topic of new conditioning regimens. In the first presentation, Dr. Pulsipher describes preclinical and clinical experience with various reduced-intensity regimens. In the second presentation, Dr. Soiffer describes his group's experience using the intravenous formulation of busulfan with fludarabine for transplantation for acute myleogenous leukemia and myelodysplasia. In the third presentation, Dr. Nagler recounts his group's experience with intravenous busulfan in ablative and nonablative regimens. In the fourth presentation, Dr. de Lima discusses his center's experience using busulfan with fludarabine and contrasts it with busulfan plus cyclophosphamide.

It's clear that innovations in conditioning regimens are spicing up allogeneic HCT. Print new menus!



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PRELIMINARY APPLICATION

Be a part of a national organization established to promote education, research, and medical development in the field of blood and marrow transplantation.

Full Membership is open to individuals holding an MD or PhD degree with demonstrated expertise in blood and marrow transplantation as evidenced by either the publication of two papers on hematopoietic stem cell transplantation–related research as recorded by curriculum vitae, or documentation of two years of experience in clinical transplantation as recorded by curriculum vitae or letter from the director of a transplant center attesting to the experience of the candidate.

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In-Training Membership is open to fellows-in-training in bone marrow transplantation programs. A letter from the transplant center director attesting to the applicant's training status is required.

Included in the membership fee is a one-year subscription to ***Biology of Blood and Marrow Transplantation*.**

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Register Online for Feb. 16-20 BMT Tandem Meetings in Hawaii

Online registration and housing reservations are open for the 2006 BMT Tandem Meetings that will be held Feb. 16-20 in Honolulu.

On a single Web page at www.asbmt.org, registrants can navigate to meeting registration, housing reservations, preliminary program, travel discounts and local tours.

Information also is provided that compares the cost of travel and lodging for Hawaii versus other convention locations.

40 Travel Grants Available for BMT Tandem Meetings

Forty travel grants of $1,000 each will be awarded to young investigators (not more than 5 years in the BMT field) submitting abstracts to the BMT Tandem Meetings.

Sessions have been designated for oral presentation of about 60 abstracts, chosen according to scores awarded by the Abstract Review Committees. All young investigators who are selected to give oral presentations will be awarded a travel grant. Application for a travel grant is automatic when young investigators submit abstracts.

Research Awards Offered for New Investigators

ASBMT has introduced two new investigator awards–one in cooperation with Protein Design Labs and the other with Astellas Pharma US.

Both awards are for $25,000 per-year, renewable for a second year. The deadline is Dec. 1.

An award application is available for download from the ASBMT Web site www.asbmt.org. Submit just the single application to be considered for both awards.

Protein Design Labs, Inc. acquired ESP Pharma, Inc. in January. Astellas was formed in April by the merger of Fujisawa Pharmaceutical Co. and Yamanouchi Pharmaceutical Co.

ASBMT and AST Launch Joint Clinical Research Award

ASBMT and the American Society of Transplantation (AST) have announced a joint Clinical Research Award for new investigators.

The award is a two-year clinical research grant, in the total amount of $80,000, paid in quarterly installments to the recipient's institution. The deadline for applications is December. 2.

The purpose of this grant is to fund research in areas that are of mutual interest to both societies, including histocompatability, immunogenetics, stem cell research and stem cell research for tolerance induction. The grant is intended for individuals initiating their careers in solid organ or blood and marrow transplantation.

Information, eligibility requirements and online application are on the AST Web site www.cmeplanning.com/ast/event/category/2. Click "AST/ASBMT Fellowship Grant."

ASBMT Membership Free for Fellows-in-Training

Post-doctoral fellows and physicians-in-training for blood and marrow transplantation are eligible for free ASBMT membership.

The annual dues of $75 is waived for new fellows-in-training in North America who join the Society. The program to recruit and waive the dues of in-training members is supported by an educational grant from ESP Pharma, a wholly owned subsidiary of Protein Design Labs.

Included in ASBMT membership is a subscription to *Biology of Blood and Marrow Transplantation*, and the bulletin, *Blood and Marrow Transplantation Reviews*. Among other membership benefits are reduced member-rate registration at the BMT Tandem Meetings and access to new investigator awards and travel grants.

Membership applications are available from the ASBMT Executive Office or by download from the ASBMT Web Site at www.asbmt.org. Click "Membership Application" on the home page.

Consortium of Groups Addressing Cell Product Coding and Labeling

Improvements in coding and labeling of cellular therapy products is the objective of a newly created consortium that includes ASBMT and 10 other national and international cell therapy organizations.

Meeting for the first time in September in Athens, the Cellular Therapy Coding and Labeling Advisory Group will promote standardization of terminology and product naming. Specifically, the Advisory Group will review existing labeling regulations, such as those of the FDA and the European Tissue and Cells Directive, and design product label templates that satisfy the regulatory requirements.

The Advisory Group is promoting the adoption of the ISBT 128 standard in cellular therapy facilities around the world, and will provide advice and support to facilities introducing the standard. It also will be advising on the further development of the ISBT 128 standard to support new therapies.

The ISBT 128 standard originated in 1994 and is being used in at least 28 countries on five continents. It provides for a donation numbering system that has a globally unique identification for each cellular product, a bar coding system for transfer of the information on the product label, a standard layout for the product label and a standard reference for use in electronic communications.

Other organizations participating in the Advisory Group are:

AABB
European Group on Blood and Marrow Transplantation
Foundation for the Accreditation of Cellular Therapy
International Council for Commonality in Blood Banking Automation, Inc.
International Society of Blood Transfusion
International Society for Cellular Therapy
ISCT Europe
JACIE (Joint Accreditation Committee ISCT-EBMT)
National Marrow Donor Program
World Marrow Donor Association

BBMT Manuscript Submission Rate Continues Multi-Year Acceleration

The pace of manuscript submissions to Biology of *Blood and Marrow Transplantation* continues to increase–from eight or nine manuscripts per month in 2002 to more than 20 manuscripts per month now.

The submission rates for the past several years have been:

2002 - 8.6 manuscripts per month
2003 - 11.8 manuscripts per month
2004 - 13.6 manuscripts per month
2005 to date - 20.4 manuscripts per month

The manuscript acceptance rate this past year was 56%.

AABB Publishes Physician Cellular Therapy Handbook

The AABB has published *Cellular Therapy: A Physician's Handbook* for medical professionals, attending physicians, house officers, students, nurses, technologists and other medical personnel.

The handbook was developed in cooperation with ASBMT, the International Society for Cellular Therapy and the National Marrow Donor Program. The editors are Drs. Edward Snyder and Rebecca Haley.

For information and online orders, visit the AABB Marketplace Web site www.aabb.org.

ASBMT

Symposium Report

Blood and Marrow TRANSPLANTATION REVIEWS

New Era of Preparative Regimens: Controlled Ablation and Reduced Toxicity

Adapted from a CME symposium presented at the American Society for Blood and Marrow Transplantation and the Center for International Blood and Marrow Transplant Research 2005 BMT Tandem Meetings, on February 11, 2005, in Keystone, Colorado.
This program is supported by an unrestricted educational grant from ESP Pharma, Inc., a wholly owned subsidiary of Protein Design Labs, Inc.

MEDICAL COLLEGE OF WISCONSIN CME program

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Robert J. Soiffer, MD
Clinical Director, Center for Hematologic Oncology, Dana Farber Cancer Institute, Boston, Massachussetts

Faculty Disclosure

As an accredited CME provider, the Medical College of Wisconsin must ensure balance, independence, objectivity, and scientific rigor in all its individual or jointly sponsored educational activities. The authors who contributed to this publication have disclosed the following relationships:

C. Fred LeMaistre, MD, has indicated that he has nothing to disclose.

Marcos de Lima, MD has indicated that he is a member of the Scientific Advisory Boad for ESP Pharma.

Arnon Nagler, MD, MSc has indicated that he has received grant support from ESP Pharma.

Michael Pulsipher, MD, has indicated that he has received grant support from ESP Pharma.

Robert J. Soiffer, MD, has indicated that he has nothing to disclose.

Continuing Medical Education Credit

The Medical College of Wisconsin is accredited by the Accreditation Council for Continuing Medical Education to provide continuing medical education for physicians.

The Medical College of Wisconsin designates this educational activity for a maximum of 1.0 category 1 credit toward the AMA Physician's Recognition Award. Each physician should claim only those credits that he/she actually spent in the educational activity. Credit is available only to those physicians who did not receive credit for attending the live program on February 11, 2005

Needs Assessment

There is a growing trend in the use of nonmyeloablative or reduced-intensity regimens (NST) prior to HSCT. In 2002, about 30% of allogeneic transplantations utilized a NST regimen. NST regimens rely on conditioning regimens in which the dose is reduced to minimize toxicities while maintaining engraftment. This has opened blood and marrow transplantation to a wider patient base that includes older patients and those with comorbid conditions who would be unable to tolerate a fully myeloablative treatment. As more transplantation centers begin to adopt this novel approach to blood and marrow transplantation further discussion of why and how certain types of regimens are currently being studied and used as part of a NST regimen is needed.

Target Audience

This program will be of value to physicians, data managers, nurses, and pharmacists who are involved in the care of recipients of blood and marrow transplants.

Learning Objectives

Upon completion of this activity, participants should be able to:

- Explain how to treat patients with a number of hematological malignancies by using a variety of reduced-intensity preparative regimens for hematopoietic cell transplantation (HCT).
- Describe how a once-daily intravenous busulfan plus fludarabine combination preparative regimen compares to busulfan plus cyclophosphamide in leukemia patients.
- Give details on how intravenous busulfan in combination with fludarabine is associated with fewer regimen-related toxicities in both related and unrelated stem cell transplantation.
- Detail the clinical studies that provide data on a once-daily intravenous busulfan plus fludarabine combination as an alternative to traditional myeloablative transplantation in patients over 50 years of age with hematologic malignancies undergoing allogeneic HCT.

Reduced-Intensity Preparative Regimens for Hematopoietic Stem Cell Transplantation

Michael Pulsipher, MD

Traditional approaches to hematopoietic stem cell transplant (HSCT) have emphasized marrow ablation with megadose chemotherapy and/or radiotherapy to achieve a cancer cure. New approaches using nonmyeloablative/reduced-intensity conditioning (RIC) regimens seek instead to achieve an appropriate immunomodulation and promote a graft-versus-tumor (GVT) effect. Targeted dosing and dosimetry associated with various chemotherapeutic and radiotherapeutic agents have added to the transplant physician's ability to specifically deliver a desired intensity of the conditioning regimens. The following discussion will review selected chemotherapeutic and radiotherapeutic methods used in nonmyeloablative/RIC transplantation conditioning, as well as the comparative efficacy of different agents in achieving immune suppression and engraftment of donor stem cells.

In the traditional model, the purpose of the stem cell infusion after megadose therapy was to rescue ablated marrow and to effect hematologic recovery. The required ingredients of this approach are myeloablation to create bone marrow space, immunosuppression to prevent rejection and graft-versus-host disease (GVHD), and disease-specific regimen to kill residual malignant cells. However, several findings led researchers to reason that T-cells of the donor would be sufficient to immunologically eradicate the cancer if donor chimerism could be established with preparative regimens emphasizing immunosuppression rather than myeloablation. Specifically, the basis for these approaches are as follows: patients with GVHD were noted to have less relapse; removal of T-cells prior to transplantation led to increased relapse; and the use of donor lymphocyte infusions (DLI) can cure certain malignancies, that have relapsed after HSCT. Finally, patients without T-cell function (severe combined immunodeficiency syndrome) could engraft without preparative regimens. Hence, the notion of reduced-intensity regimens for transplantation was born.

The rationale of nonmyeloablative/RIC HSCT is to establish donor chimerism that allows alloimmune tumor kill. Full myeloablation is no longer needed to create bone marrow space. Disease-specific chemotherapeutic agents are not necessarily required either because the new marrow cures through immunologic attack of the cancer. Immunosuppression is still necessary, however, to prevent rejection and to control GVHD. Three key considerations in choosing RIC regimens are (1) understanding the immunological status of the patient, (2) knowing the susceptibility of the underlying disease state to immunologic therapy, and (3) estimating the rate at which the underlying cancer is progressing (rapidly progressing cancers do not allow time for immunologic effect). The immune status of the patient is also important when devising a preparative regimen. Patients with an intact immune system will require more immunosuppression than those who have received prior chemotherapy. In addition, the status of remission and the type of malignancy are important because patients with unstable cancer or incomplete remission may need more intensive therapy prior to RIC regimens to create enough time for the allogeneic immune response to occur. Over the last few years, a wide variety of preparative regimens have been developed, each with a different amount of immune suppression, myeloablation, and ability to control a given malignancy. Fully myeloablative regimens provide maximal immune suppression and myeloablation with intense cancer control. Intermediate-intensity regimens provide some cancer control and myeloablation with moderate/intense immune suppression, and minimal-intensity or nonmyeloablative regimens provide mild/moderate cancer control and moderate immune suppression.

Studies have shown that 3 variables of the reduced-intensity HSCT process can be modulated in ways that are important in achieving successful transplantation outcomes. The variables are (1) the immune status of the patient prior to the transplantation, (2) the agents used in the RIC preparative regimen, and (3) the type and timing of immune suppressive medications used after transplantation. To manipulate the patient's immune system prior to transplantation, investigators at the National Institutes of Health used an approach of administering a series of lymphocyte depleting chemotherapy prior to allogeneic transplantation until the recipient's absolute CD4 count reached below 0.05×10^9cells/L. Patients then received a minimally intensive RIC regimen (fludarabine/cyclophosphamide) followed by ex vivo T-cell depleted human leukocyte antigen-matched sibling allografts. Full donor chimerism was achieved in all patients after planned DLI. These results showed the importance of host immune status prior to nonmyeloablative therapy, and suggest that targeted host lymphocyte depletion facilitates the engraftment of T-cell–depleted allografts [1].

The first published reports using reduced-intensity regimens for hematologic malignancies came from the M.D. Anderson Cancer Center (MDACC) in Houston and the Hadassah University in Jerusalem. Both groups capitalized on the intensely T-cell–immunosuppressive properties of fludarabine, but differed otherwise in their fundamental approach. The MDACC included agents with known activity in specific malignancies, for example, combining fludarabine with idarubicin and cytosine arabinoside for AML [2-5], and the Hadassah group used a single regimen of intense immunosuppression for a wide variety of hematologic diseases [6]. The latter approach included fludarabine (30 mg/m^2 per day for 6 consecutive days), anti–T-lymphocyte globulin (10 mg/kg/day for 4 consecutive days), and low-dose oral busulfan (4 mg/kg per day for 2 consecutive days) and depended on alloreactive cells both for engraftment and potentially for tumor response. The regimen was extremely well tolerated, with no severe procedure-related toxicity. Early results showed that with an observation period extending more than 1 year (median 8 months), 22 (85%) of 26 patients were alive, and 21 (81%) were disease free. The actuarial probability of disease-free survival at 14 months was 77.5%. Thus, successful elimination of malignant and genetically abnormal host hematopoietic cells by allogeneic nonmyeloablative stem cell transplantation represents a potential new approach for safer treatment of a large variety of clinical syndromes with an indication for allogeneic BMT. The major challenge with the approaches from MDACC and the Hadassah group is a high rate of acute and chronic GVHD. Because the allogeneic GVT effect is usually associated with GVHD, however, outcomes of patients with moderate acute and chronic GVHD have been better than those with severe GVHD (more transplant-related mortality) or no GVHD (more relapse).

Another approach from investigators in Great Britain attempted to decrease rates of GVHD by using alemtuzumab, a monoclonal antibody that causes significant T- and NK-cell depletion. The initial publication detailed outcomes of 44 patients using a nonmyeloablative conditioning regimen consisting of alemtuzumab, fludarabine, and melphalan followed by infusion of peripheral blood stem cells from HLA-identical siblings (n = 36), with 8 receiving unmanipulated marrow from matched unrelated donors [7]. GVHD prophylaxis was accomplished with cyclosporine (Csa) alone for 38 patients and Csa plus methotrexate for 6 recipients. The results showed that 42 of the 43 evaluable patients had sustained engraftment. At a median follow-up of 9 months

(range, 3 to 29 months), 33 patients remained alive in complete remission or with no evidence of disease progression. Seven patients relapsed or progressed posttransplantation, and 4 of them subsequently died. Four patients died of regimen-related complications. There were no cases of grade III-IV acute GVHD. Follow-up studies using this method have shown very high rates of CMV reactivation and other significant infections, and slow recovery of immune function. The study demonstrated that using reduced-intensity and immunoblative preparative regimen is associated with durable engraftment and a low incidence of GVHD. Which of these approaches (more GVHD and less relapse risk or less GVHD and more infection risk) is better is not known, because randomized comparative studies of the different regimens have not been performed.

To understand posttransplantation immune suppression and how it affects engraftment and outcome, investigators in Seattle used a canine transplantation model to define the lowest doses of immune modulating agents needed to establish long-term donor chimerism. A total-body irradiation (TBI) dose of 920 cGy without posttransplantation immune suppression was sufficient for sustained donor chimerism, but when the dose of TBI was decreased by 50%, most of the dogs rejected. Adding prednisone after the transplantation using the lower TBI dose did not increase engraftment, but the addition of cyclosporine led to full engraftment in all cases. When the TBI was lowered to 200 cGy, adding Csa alone resulted in only transient engraftment, with rejection by day +30. The addition of mycophenylate mofetil (MMF) for the first 4 weeks after transplantation, however, led to long-term engraftment in 11 of 12 dogs. Further lowering of the TBI dose to 100cGy led to rejection, showing that the group had truly defined the minimal amount of immune suppression needed to establish chimerism in this model [8].

This canine study led to an approach used by many transplantation institutions where patients were given minimal intensity preparations followed by transplantation. In an early study of 45 patients with various hematologic malignancies, published by the Seattle group, immunosuppression with TBI at 200 cGy before and Csa/MMF after HSCT was used [9]. In addition, fludarabine 30 mg/m^2 was administered on days –4, –3, and –2 prior to HSCT in an attempt to reduce the risk of graft rejection. The results showed that this novel allografting approach, based on the use of postgrafting immunosuppression to control graft rejection and GVHD, dramatically reduced the acute toxicities associated with allografting. In addition, by focusing on postgrafting immunosuppression to control rejection and GVHD, there was an additional reduction of the intensity and toxicity of conditioning therapy for recipients of HLA-identical grafts.

The Seattle canine studies elegantly showed the relative abilities of TBI, cyclosporine, and MMF to foster engraftment in the nonmyeloablative transplantation setting. Such studies were possible because of the dose assurance and consistent delivery of TBI, to allow evaluation of specific outcome parameters. However, not all patients are candidates for TBI and thus a chemotherapy-based approach is needed. Busulfan (Bu) has been most commonly used as an alternative to TBI. The availability of an intravenous (IV) formulation of Bu allows for 100% bioavailability and predictable pharmacokinetics of busulfan that provide dose assurance and the ability to accurately deliver varying doses/fractions, depending on desired outcome level of myeloablation. Iannone and Fuchs [unpublished data, 2005] investigated the use of IV Bu at Johns Hopkins using a mouse model to compare myeloid chimerism after conditioning with various doses of IV Bu or similar fractions of TBI, given alone or in combination with cyclophosphamide posttransplantation. Mice were conditioned with 10, 20, or 30 mg/kg IV Bu given on each of 2 days or 100, 300, 500 or 700 cGy of TBI given once. Using a congenic mouse model (equivalent to syngeneic twins), engraftment, defined by donor myeloid chimerism, occurs with as little as 20 mg/kg of IV Bu or 300 cGy TBI conditioning. H2 compatible, minor MHC incompatible mice require 40mg/kg IV Bu or 550cGy TBI for engraftment. H2 and minor MHC incompatible mice (equivalent to human HLA identical sibling transplants) require 60 mg of IV Bu or 700 cGy TBI to allow the mice to establish donor chimerism. The addition of posttransplantation cyclophosphamide allowed lower doses of either IV Bu or TBI to be given. H2 and minor MHC incompatible mice receiving 40 mg/kg IV Bu or 550 cGy of TBI when they receive a single dose of cyclophosphamide at day +2 achieved 80% to 90% donor engraftment.

In summary, reduced-intensity/nonmyeloablative approaches have changed the way transplantation physicians approach patients, allowing less toxic regimens that deliver immunotherapy to patients previously ineligible for transplantation (older patients or patients ineligible for standard transplantation). A wide variety of approaches have targeted pretransplantation immune suppression of the patient, assessed various intensity levels of chemotherapeutic/radiotherapeutic involvement with transplantation preparative regimens, and investigated novel ways to use posttransplantation immune suppression to promote engraftment. Pharmacokinetic measurement of systemic exposure of chemotherapeutic agents allows for more targeted therapy in this patient population. Accurate dosimetry of either chemotherapy or radiotherapy will allow therapy to increase engraftment and potentially decrease both acute and long-term toxicities. Which approaches are the best for any given patient are not yet known, awaiting comparative trials of reduced-intensity approaches or trials comparing RIC to standard transplantation regimens. In the future, HSCT will likely employ targeted, nonoverlapping therapies to achieve engraftment and disease control. Promising novel HSCT strategies currently being studied that may come to more prominence include antibodies that interfere with co-stimulatory pathways in allo-reactive T-cells, novel immunosuppressive agents that cause apoptosis of activated allo-reactive T-cells, stem cell–sparing cytotoxic agents after HSCT to kill differentially allo-reactive T-cells, novel approaches taking advantage of KIR (killer immunoglobulin–like receptor) incompatibility, and manipulation of donor T-cells with a T-regulatory phenotype.

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Novel Uses of Intravenous Busulfan for Nonmyeloablative Allogeneic Stem Cell Transplantation

Robert Soiffer, MD

The following is a brief summary of one of our nonmyeloablative transplant recipients. In August 2003, a 55-year-old woman with acute myelogenous leukemia (AML) in complete remission 2 (CR2) underwent a myeloablative allogeneic peripheral blood stem cell transplantation (PBSCT) obtained from her HLA identical sister. The patient did well with no graft-versus-host disease (GVHD). In January 2004, the original donor suffered a myocardial infarction and underwent coronary artery bypass surgery, and the other HLA identical sister was discovered to have breast cancer. In February 2004, the AML patient relapsed and received salvage chemotherapy that produced a partial remission (20% blasts in the marrow). The patient's first cousin was found to be an HLA identical match, so in March 2004 a nonmyeloablative allogeneic transplantation from her cousin was performed using a combination of intravenous busulfan (IV Bu) and fludarabine (Flu) as conditioning regimen. As of February 2005, the patient is alive and in remission with 100% donor cells 11 months later.

Our collective experience from nonmyeloablative allogeneic stem cell transplantation (NST) suggests that engraftment can generally be achieved with low levels of early toxicity, which allows transplantation for older patients and those with medical contraindications to high-dose conditioning therapy. GVHD occurs at a later time than conventional myeloablative transplantation, but perhaps with equal incidence. It remains unclear whether nonmyeloablative (reduced-intensity or "mini") transplantation is superior to conventional myeloablative transplantation. According to the CIBMTR, the number of nonmyeloablative transplantations has markedly increased from 1997 (20 cases) to 2001 (870 cases). There are critical issues to consider before employing nonmyeloablative transplantation; for example, conditioning should be minimally toxic, provide reliable and reproducible levels of active agent, and facilitate a high degree of donor hematopoietic chimerism.

In AML patients in particular, it is important to determine the contributions of conditioning-dose intensity and graft-versus-leukemia (GVL)/alloreactivity to cure. It is known that autologous HSCT can result in long-term cures for patients with AML in second remission, whereas it is unlikely that chemotherapy alone can do the same. High-dose cytarabine (ara-C), is more effective than intermediate or low-dose ara-C in preventing relapse for patients <60 years of age with AML in first remission. In a Cancer and Leukemia Group B (CALGB) study [1], ara-C was found to produce greater survival benefits in AML patients <60 years of age at high doses of 3 g/m^2 as compared to lower doses of 400 or 100 mg/m^2 up to 84 months.

However, there is evidence that challenges the notion that dose intensity is critical in AML. Most studies have shown at most a minimal benefit of autologous transplantation compared with chemotherapy for patients with AML in CR1. Benefits of dose-intensive therapy likely depend on disease-specific factors such as age and cytogenetics. In the CALGB trial there was no benefit in giving high-dose ara-C to older individuals. Overall survivals of recipients of 3 g/m^2, 400 mg/m^2, and 100mg/m^2 were equivalent. In a subset analysis of the ECOG randomized trial of chemotherapy versus transplantation as postremission treatment for AML in first remission, a regimen of high-dose therapy and autologous transplantation was superior to chemotherapy only for patients with favorable cytogenetics at presentation, not for patients with an adverse karyotype [2].

A survey of the CIBMTR and ASBMTR registries showed that NST regimens for allografting have been predominantly employed in patients between 50 and 70 years of age. There are multiple nonmyeloablative regimens in use today. They differ in their myelosuppressive and immunesuppressive properties. It remains unclear which regimen, if any, is superior. At Dana Farber Cancer Institute, we have utilized IV Bu at 0.8 mg/kg per day × 4 days with Flu at 30 mg/m^2 per day × 4 days, a nonmyeloablative regimen.

We carried out a retrospective study in older patients (>50 yrs) comparing our experience with our nonmyeloablative regimen (IV Bu Flu) to standard Cy/TBI conditioning in patients with a variety of hematologic malignancies [3]. Incidence of grades II-IV GVHD was no different in these patients receiving myeloablative or nonmyeloablative regimens. Survival of these older patients was at least as good after nonmyeloablative transplantation as after fully ablative transplantation. Not surprisingly, cause of failure differed in the 2 groups; relapse was higher in the NST group, whereas treatment related mortality was greater in the ablative cohort [3].

We conducted a similar retrospective analysis specifically in AML and MDS patients comparing NST and myeloablative conditioning regimens. Again, the design was retrospective, but this time included patients of all ages. Mortality at day 100 was lower in NST patients compared to myeloablative patients. There was no significant difference in overall survival or progression-free survival for the groups at 1 year, though there was a suggestion of superiority for the myeloablative group at 2 years. However, when the cohorts were again broken down into those above and below the age of 50, the advantage for myeloablative transplantation lay completely in the under-50 group. In patients over 50 years of age, NST results were at least as good as those with myeloablative conditioning.

The use of reduced-intensity conditioning leads to the question of donor chimerism, whether levels of donor hematopoietic cell chimerism after transplantation influence outcomes including graft rejection, GVHD, and relapse. The impact of early donor chimerism on outcome has yet to be determined, but likely depends on disease, extent of marrow involvement, and prior treatment. We compared early donor chimerism in patients with AML or MDS achieving a high degree of donor chimerism (>90%) at ~1 month after nonmyeloablative transplantation with patients achieving <90% donor-derived hematopoiesis [4]. The impact of hematopoietic chimerism on outcome after NST was reported in 64 patients with AML or MDS using IV Bu and Flu conditioning regimen. The overall survival after 1 year in the >90% donor chimerism patients was 71% and for <90% donor chimerism patients it was 39%. One-year progression-free survival was 41% for >90% donor and 21% for <90% donor patients. The development

of GVHD was similar in both groups (19%). Overall survival and progression-free survival after NST was greater in >90 chimerism patients than in the <90 chimerism patients after more than 2 years [4]. There was little difference in the cumulative incidence of treatment-related mortality. The cumulative incidence of the risk of relapse was greater in the patients with <90 donor chimerism.

In conclusion, NST is associated with a relatively low treatment-related mortality in high-risk patients with AML and MDS. Despite the adverse features of the NST population, for patients over 50 years of age OS and progression-free survival were similar to patients undergoing myeloablative transplantation. Achieving a high level of donor chimerism (>90%) early after NST is associated with improved overall survival in patients with AML and MDS. The primary reason for treatment failure for patients achieving <90% donor chimerism is relapse of disease. The degree of donor chimerism achieved after NST may reflect the presence of a graft-versus-host marrow effect and becomes a surrogate marker for a GVL effect. It remains to be determined if the augmentation of donor chimerism will alter the risk of relapse and outcome after NST.

Future research directions for nonmyeloablative transplantation are to reduce the risk of chronic GVHD, improve GVL activity, and improve tumor-specific immune response.

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Myeloablative Conditioning Using IV Busulfan Reduces Toxicity to the Level Observed in Low-Intensity Conditioning Regimens: Dose Does Matter

Arnon Nagler, MD, MSc

There is evidence that in AML patients myeloablative doses of chemotherapy can be administered along with reduced toxicity, using intravenous busulfan (IV Bu), and that dose does matter in patient outcome.

Standard allogeneic hemopoietic stem cell transplantation (alloSCT) is an effective, potentially curative treatment of advanced or high-risk hematological malignancies. The procedure is associated with significant morbidity and mortality because of the toxicity of the conditioning regimen, graft-versus-host disease (GVHD), and immune-deficiency state that accompany the procedure. These risks substantially increase with advanced age, comorbidities, or extensive prior therapy. Long-term side effects, including sterility, growth retardation, and endocrine problems are also considerable.

Over the last decade, nonmyeloablative stem cell transplantation (NST) and reduced-intensity conditioning (RIC) regimens have been designed to reduce toxicity, including long-term effects, and allow successful alloSCT in elderly and medically infirm patients. These regimens are designed not to maximally eradicate the malignancy, but rather to provide sufficient immune suppression to achieve engraftment and to allow induction of graft-versus-leukemia (GVL) effect as the primary treatment. After transplantation, immune interventions are often required to hasten the GVL effect. However, relapses of the basic disease and GVHD remain major challenges for successful RIC allogeneic HSCT, especially in patients with advanced disease and high-grade malignancies.

First-Generation RIC and Nonmyeloablative Conditioning Regimens

The first generation of nonmyeloablative conditioning regimens consisted of a spectrum of drugs with different immunosuppressive and myelosuppressive properties. These included the following prototypical combinations: total body irradiation (TBI) (200 cGy)/cyclosporine A (Csa)/MMF or FLAG/Ida. The 2 combinations do not completely eradicate host hematopoiesis and immunity. They are only mildly myelosuppressive and usually induce mixed chimerism (MC), while complete chimerism (CC) and GVL may develop slowly, spontaneously, or after immune intervention.

Additional RIC regimens included Flu/oral Bu/ATG or Flu/Mel 200/alemtuzumab and have not been administered without stem cell support. Autologous (auto) recovery may be slow, if at all. These regimens are myeloablative, rapidly inducing CC and anti-tumor responses and are less toxic than the standard pre-alloSCT conditioning. The auto/allo double stem approach is high-dose chemotherapy and autoSCT followed by RIC or NST alloSCT, and this approach is being used frequently to treat multiple myeloma [1]. In the selection of the optimal conditioning regimen, several factors have to be considered, such as the patient's age and general medical condition, recipient immune competence, donor source, and experience of the center. NST conditioning should be used in elderly and sick patients and should be considered after determining the immunocompetence of the patient and the donor source. Additional factors are the aggressiveness and chemo-susceptibility of the underlying malignancy and the underlying malignancy susceptibility to the GVL effect [1].

Conditioning Agents

Intravenous Busulfan

Bu/Cy is a widely used regimen for myeloablative conditioning. Oral Bu is also used in low-intensity conditioning regimens. Oral busulfan (Bu) has an erratic and unpredictable absorption with wide inter- and intra-patient variability. A high area under the curve of Bu blood concentration-versus-time is associated with a high risk of veno-occlusive disease (VOD). Conversely, low Bu concentrations are associated with a higher risk of graft rejection and leukemia relapse. Monitoring Bu concentrations and dose adjustments can allow better control of the dose administered and reduction of these risks, but such results cannot be easily achieved in many patients. To obviate these risks, IV Bu was recently introduced into clinical use. Absorption is more predictable with less need for PK testing and dose adjustments and a more favorable and predictable toxicity profile.

A clinical study was carried out to define the role of IV Bu in different transplantation and dis-

ease settings to depict myeloablation along with reduced toxicity in successful transplant recipients [2]. Forty-eight patients (32 adults and 16 children) with a median age of 30 years (range, 1 to 58) were enrolled in the study. There were 38 patients with myeloid disease, 7 with lymphoid disease, and 3 nonmalignant patients: AML (n = 28) (9 secondary malignancies), MDS (n = 5), CML (n = 4), myelofibrosis (n = 1), ALL (n = 6), NHL (n = 1), thalassemia (n = 2), and FHL (n= 1).

There were 2 preparative regimens utilized. The myeloablative regimen consisted of:

- IV Bu 0.8 mg/kg ideal body weight given in 16 doses on days –7 to –4;
- IV Cy 60 mg/kg given in 2 doses on days –3 to –2;
- Patients with MUD were given ATG IV 5 mg/kg in 2-3 doses.

The RIC regimen consisted of:

- Flu 30 mg/kg IV on days –6 to –2;
- IV Bu 0.8 mg/kg IV given in 12-16 doses on days –5 to –2;
- ATG IV 5 mg/kg given in 4 doses on days –4 to –1 (optional).

All patients received their peripheral blood stem cell infusion on day 0 ± day 1. For GVHD prevention, each patient received CSA+ short course MTX (days 1, 3, and 6).

The results showed that there was no significant difference (P >.05) between nonmyeloablative and myeloablative conditioning regimens in the incidence of TRM in patients after 2.5 years of transplantation. There was no difference in DFS between the 2 regimens either. It was concluded that regimens containing IV Bu allow consistent engraftment of allografts from both related and unrelated donors, and that there was an improved toxicity profile in comparison with the historical use of oral Bu. IV Bu provides reliable intrapatient and interpatient consistency. The use of the IV formulation allows for predictable systemic exposure without drug monitoring. In particular, there was a low risk for VOD (only 1 patient) and no excess risk to other organs, such as the lungs or CNS. Nonrelapse mortality and short-term outcome seemed to be improving, in particular, in high-risk transplantations, such as those from unrelated and mismatched donors. The toxicity profile resembled that achieved with reduced-intensity regimens [2].

Fludarabine/IV Busulfan versus Fludarabine/Melphalan

A clinical study was conducted to compare the efficacy and safety of 2 reduced-intensity conditioning regimens administered prior to allogeneic HSCT. One hundred patients were enrolled. There were 59 men and 41 women, with a median age of 53 years (range, 20 to 66 years). Hematological malignancies consisted of AML (n = 27), MM (n = 30), ALL (n = 2), CLL (n = 4), MDS (n = 5), CML (n = 6), NHL (n = 20) and HD (n = 6). Transplant donors represented matched siblings (n = 58) and matched unrelated (n = 42). Based on the results in the patients treated, approximately 2.5 years after HSCT, the TRM following the Flu IV Bu regimen was significantly less (P = .01) than that observed following the Flu Mel combination conditioning regimen, and OS in patients with myeloid malignancies presented similar results. Thus, the combination of Flu and IV Bu according to multivariate analyses had a more favorable toxicity profile and overall survival outcome (P = .05) than the Flu Mel combination as a reduced-intensity preparative regimen prior to allogeneic HSCT. Overall survival was better with IV Bu Flu than with Flu Mel. Thus, the toxicity profile and other outcomes are more favorable with IV Bu Flu than Flu Mel as a conditioning regimen for allogeneic transplantation [3].

Table. The Toxicity Profile According to the Bearman Scale (N = 48)

	Number of Patients (%)
Mucositis grade I-II	Most
Jaundice grade I-II (transient in all)	18 (37)
Veno-oclusive disease (resolved)	1 (2)
Hemorrhagic cystitis frade I-II	5 (10)
Cardiac toxicity grade II-III	2 (4)
Severe TTP	2 (4)
Renal toxicity grade II-III	1 (2)
Pneumonitis grade IV	1 (2)
Day +100 treatment-related toxicity related to organ toxicity	1 (2)

Prospective Clinical Trial

A prospective study was carried out in patients with chemo-sensitive AML and MDS. They were not eligible for standard conditioning and underwent alloSCT using Flu and either myeloablative (4 days) or reduced-intensity doses (2 days) of IV Bu. OS and DFS after transplantation of 10 months or more were significantly greater (P = .05) when the patients received IV Bu for 4 days rather than 2 days. There were no significant differences (P >.05) in TRM between the 2 groups. Relapse rate was significantly greater (P = .05) in the IV Bu patients on the 2 day-therapy (80%) rather than on the 4-day therapy (34%). Thus, allogeneic HSCT with reduced-intensity IV Bu was not effective in patients with refractory disease or untreated disease with >10% marrow blasts at SCT due to very high incidence of relapse. Patients with refractory disease can be salvaged with myeloablative doses of IV Bu, and patients considered not eligible for standard myeloablative conditioning (Bu Cy) could tolerate Flu and ablative doses of IV Bu with limited TRM and acceptable outcome.

In patients with sensitive disease undergoing allogeneic HSCT and receiving either myeloablative (4 days) or reduced-intensity conditioning (2 days) doses of IV Bu, the OS and DFS in 43 patients at >40 months transplantation was not significantly different (P >.05): OS 75% for 2 days versus 55% for 4 days, DFS 70% for 2 days versus 54% for 4 days. There was no difference in TRM (nonrelapse mortality) and relapse rate between the 2 groups. Thus, allogeneic HSCT with myeloablative and reduced-intensity IV Bu are equally effective in patients with chemo-sensitive disease or relapsed disease with <10% marrow blasts at transplantation. TRM is very low when reduced doses are administered, and relapse is not increased despite selection of older patients with poorer general conditioning for reduced intensity.

Overall conclusions regarding IV Bu use for alloSCT are that the use of IV Bu is associated with fewer regimen-related toxicities in both related and unrelated stem cell transplantation [3]. The Flu IV Bu regimen has a better toxicity profile in comparison to the Flu Mel regimen. Flu IV Bu × 4 days results in better disease control compared to Flu IV Bu × 2 days in leukemia patients with active disease (resistant or >10% marrow blasts), and both regimens are equally effective in patients with sensitive disease (in remission or <10% marrow blasts). Thus, dose in AML does matter.

Future research directions include new strategies against GVHD (halofuginone, oral tolerance), tumor-specific DLI following T-cell–depleted grafts, targeted therapy, tumor vaccination, improved immune reconstitution, and CMV- and Aspergillosis-specific CTLs [1].

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Treatment of AML/MDS with Allogeneic Hematopoietic Stem Cell Transplantation after Intravenous Busulfan-Based Conditioning Therapy

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Background

Reliable and reproducible delivery of myeloablative conditioning therapy preceding hemopoietic stem cell transplantation (HSCT) is of utmost importance for optimizing the safety of the procedure. The use of oral busulfan (Bu) in this setting is fraught with the risks imposed by the unpredictable and erratic bioavailability of the oral drug formulation. The irreproducible bioavailability of oral Bu stimulated the design of an intravenous (IV) Bu formulation that allows 100% dose assurance in delivery [1,2]. Nonrandomized comparisons indicated that patients treated with IV Bu in combination with cyclophosphamide (Cy) experience a lower incidence of veno-occlusive disease of the liver (VOD) and other serious toxicities than comparable patients receiving the oral Bu-Cy combination [3,4]. However, further information also indicates that activated Cy metabolites have a major contributory, causative role in the manifestation of VOD and other serious toxicities [5]. The presumed mechanism for this is through consumption of glutathione (GSH). The highly immunosuppressive nucleoside analog fludarabine (Flu) is metabolically deactivated through deamination without a need for GSH. We therefore postulated that the substitution of Cy with Flu would further improve the safety of the pretransplantation conditioning regimen, and maintain or even improve anti-leukemic activity. Furthermore, Flu has a very long half-life, and with the use of IV Bu and Flu together, it would be convenient to administer both drugs as a once-daily infusion. Optimal sequencing of drug administrations would take advantage of a purported synergy in DNA-damage repair inhibition between nucleoside analogs and bifunctional DNA alkylating agents [6]. In addition, a higher systemic Bu peak concentration can be achieved without excessive toxicity, which may be an added advantage when patients undergo transplantation for leukemia, by allowing better penetration of "sanctuary sites" [7,8].

Our preliminary results using the IV Bu-Flu regimen as myeloablative pretransplantation conditioning therapy in patients with myeloid leukemia (Table 1), suggest that this combination is safe and provides adequate immunosuppression for reproducible, sustained donor cell engraftment [8]. We recently reported data from 96 patients who received transplants for high-risk acute myelogenous leukemia (AML) and myelodysplastic syndrome (MDS) using this schedule. Donors were HLA-compatible related (n = 60) or unrelated (n = 36). The complete remission (CR) rate for 54 patients with active disease at transplantation was 85%. One-year regimen-related and treatment-related mortalities were 1% and 3%, respectively, with a median follow-up of 12 months. Reversible hepatic VOD occurred in 2 cases. Actuarial 1-year overall and event-free survival were 65% and 52% for all patients, and 81% and 75% for patients undergoing transplantation in any form of CR. Recipient age and donor type did not influence survival. Our data suggest that once-daily IV Bu-Flu is a safer pretransplantation conditioning regimen than the IV Bu-Cy2 combination for allogeneic HSCT.

Table 1. Pretransplantation Conditioning Regimens

Intravenous Busulfan/Cyclophosphamide (Bu/Cy)								
Day	1	2	3	4	5	6	7	8
Bu 0.8 mg/Kg every 6 hours x 16 doses	*	*	*	*				
Cy 60 mg/Kg once daily × 2					* +	* +	Rest +	HSCT

ATG if MUD or 1-antigen mismatched related donor.

Intravenous Busulfan/Fludarabine (Bu/Flu)							
Day	1	2	3	4	5	6	7
Bu 130 mg/m^2 daily × 4	*	*	*	*			
Flu 40 mg/m^2 daily × 4	*	*	*	* +	Rest +	Rest +	HSCT

GVHD prophylaxis in both protocols were: tacrolimus and "mini" methotrexate + day of ATG if MUD or 1 antigen mismatched related donor.
Fludarabine is given as a 60 min infusion, each dose immediately followed by IV Busulfan 130 mg/m^2 over 3 hours. Both drugs are administered by controlled rate infusion by pump.

We then compared 2 IV Bu-based consecutive protocols to determine whether once-daily IV Bu-Flu was safer than the IV Bu-Cy2 regimen with IV Bu given every 6 hours for 16 doses. The outcomes of interest were 1-year and 100-day treatment-related mortality, as well as event-free and overall survival. High-risk patients were treated on protocols with analogous eligibility criteria, as follows: AML past first remission or induction failure, AML in first remission without good prognosis cytogenetics, myelodysplastic syndromes with high IPSS (≥2) score, age 13 to 65 years, with a ZUBROD performance status <2, HIV negative, adequate normal organ function, and no evidence of hepatitis. Donors were related HLA-identical, 1 antigen mismatched, or matched unrelated (MUD). Table 2 presents the characteristics of the patients enrolled in the study.

Although the proportion of patients in CR at HSCT was similar in both groups, patients treated with Bu-Cy were younger and had a smaller proportion of patients that received an unrelated donor transplant than the group treated with the Bu-Flu regimen. Older age and transplants received from donors other than HLA-identical siblings are variables that usually are associated with a less favorable outcome after allogeneic HSCT, and the observed differences in this comparison would therefore favor the Bu-Cy subgroup.

Results

NRM at day +100 following transplantation was 7% in the Bu-Cy group and 2% in the Bu-Flu group. Overall and event-free survival (OS and EFS, respectively) was improved for patients in CR at the start of pretransplantation chemotherapy and treated with once-daily IV Bu and Flu. By contrast, there was no significant difference in EFS and OS for patients undergoing transplantation with active disease when comparing the 2 conditioning regimens.

Recurrence or persistence of leukemia was the most frequent cause of death in both the Bu-Cy and the Bu-Flu patients. The conclusions of this comparison were that both IV Bu ablative regimens are safe, and that IV Bu and Flu likely provides better event-free and overall survival for patients in CR at transplantation.

The pharmacokinetic (PK) parameters of IV Bu were investigated in patients receiving either a daily dose of 130 mg/m^2 (n = 60) or 0.8 mg/kg every 6 hours for 16 doses (n = 47), as described above. With the once-daily dose of IV Bu, the median area under the curve (AUC) of plasma busulfan concentration was 4925 μMol-min versus 1292 μMol-min per dose when Bu was given every 6 hours [9]. Approximately 10% of the patients treated with once-daily Bu had AUCs in excess of 6,000 μMol-min, without experiencing additional clinical toxicity.

Based on these findings, we now hypothesize that it should be possible to safely target an average daily AUC of approximately 6,500 μMol-min over the 4-day treatment course to improve the treatment results for patients undergoing transplantation with high-risk disease, given that although both IV Bu-based regimens were

Table 2. Patient Characteristics

Patients – Donors	Bu Cy, n = 72	Bu Flu, n = 128	*P*
Disease status at transplantation			
First-third CR	34 (47%)	60 (47%)	
Active disease	38 (53%)	68 (53%)	
Donor			
HLA identical related	56 (78%)	72 (56%)	.02
1-Antigen mismatched sibling	4 (6%)	6 (5%)	
Unrelated donor	11 (15%)	50 (39%)	.0004
Syngeneic	1 (1%)		
Median age (years)	39 (range, 13-64)	46 (range, 19-66)	.04
Median follow-up (months)	47 (range, 24-76) n = 26 alive	17.4 (range, 7.5-42) n = 76 alive	

demonstrated as safe, outcomes of patients with active disease at HSCT (approximately 20% 1-year EFS) were similar with both the IV Bu-Cy2 and the IV Bu-Flu regimens.

Based on previously reported observations with busulfan-based pretransplantation conditioning regimens, a low systemic drug exposure may yield higher rates of disease relapse and graft rejection. In contrast, a high AUC correlates with high/excessive toxicity and treatment-related mortality. Therefore, a therapeutic interval can be postulated to exist, covering a systemic exposure, represented by the AUC, that will promote both safe engraftment and optimized anti-leukemic activity, but without significantly increasing clinical regimen-related toxicity. Based on this hypothesis, a phase IIb trial of IV Bu-Flu in AML/MDS patients undergoing HSCT is being conducted with the goal to target an average daily AUC of 6500 μMol-min (±700) over the 4 days of Bu administration. Flu 40 mg/m^2 is administered on the same 4 days as IV Bu, each Flu dose immediately preceding the IV Bu dose, analogous to the regimen used in the previous study [8]. A test dose of Bu (32 mg/m^2) is administered 48 hours prior to starting the actual high-dose regimen. This lower test dose is used to determine the individual PK parameters that are utilized for calculating the therapeutic daily Bu dose. A "quality-control" series of Bu PK samples is also drawn and used to determine the first and third day PK parameters of the high-dose IV Bu administration. On the 2 rest days before HSCT, ATG is administered if the donor is MUD or related 1-Ag mm. Bone marrow or PBPC is infused on day 9; in other words, there are 2 rest days between the last day of Bu and Flu and the HSCT (analogous to Table 1) .

To date, we have treated 24 high-risk patients; 20 of them are alive in CR with a median follow-up of 6 months (range, 2-13 months). Three patients died, 1 of TTP/HUS (6 months), 1 of fungal pneumonia (6 weeks), and 1 of hepatorenal failure/multi-organ failure (3 months). The PK-directed individualized dose adjustments allowed all patients to achieve a targeted AUC of 6500 μg/Mol-min (±400 μMol-min).

Acute toxicities have been limited mainly to reversible gastrointestinal and hepatic impairment. Not surprisingly, the approximately 30% augmented average intensity in delivered IV Bu dose significantly increased the incidence and severity of mucositis when compared to the initial experience with the regimen reported in the previous study [8].

Our preliminary experience with this approach indicates that the use of PK guidance to deliver individualized myeloablative conditioning therapy is safe, but labor-intensive. The data suggest that this strategy deserves further investigation, most notably in high-risk patients with active disease, ie, those with relapsed and refractory AML and MDS.

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Case Study: Reduced-Intensity Preparative Regimen

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A 67-year-old woman with Behçet's disease, Raynaud's syndrome, and acute myelogenous leukemia (AML) was referred for hemopoietic stem cell transplantation (HSCT). In 1993, she was treated with hydroxyurea for polycythemia vera. In February 2002, she presented with AML with the trisomy 8 cytogenetic abnormality. Trisomy 8 is the most frequent numerical chromosome aberration in AML. It occurs either as the sole anomaly or together with other clonal chromosome aberrations. This patient received a typical therapy of "3 and 7" remission induction. The first remission consolidation therapy consisted of idarubicin and cytarabine and the second and third consolidations utilized mitoxantrone and cytarabine.

Cytogenetic clinical remission was demonstrated in July 2002. The patient was told that she was too old for transplantation. However, in October 2003 she experienced a cytogenetic relapse and second opinions were obtained at cancer clinics in Houston and Seattle. In February 2004, a hematologic relapse occurred with signs of significant myelofibrosis. Megakaryocytes were also present. Blasts stained for CD34 and CD33 but not for CD61. In March 2004, reinduction with fludarabine, cytarabine, and idarubicin was accomplished and in April of 2004 a reduced-intensity transplantation was performed using a matching unrelated donor (MUD). HSCT was performed using a reduced moderate-intensity

preconditioning regimen consisting of intravenous oral busulfan (Bu) 3.2 mg/kg q day ×2 with fludarabine (Flu) 30 mg/m^2 q day ×5. On day 0, 7.6 × 10^6 CD34 cells/kg peripheral blood progentior count were infused and graft-versus-host disease prophylaxis was tacrolimus and short course (3 days) methotrexate.

In this high-risk AML patient, engraftment (absolute neutrophil count of 500) occurred on day 12. Chimerism at day 30 was 95% donor and at day 60 there was 100% donor chimerism. Rheumatoid factor cleared over the first 60 days. The patient experienced 1 day of fever but there were no reports of nausea, mucositis, or veno-occlusive disease.

This case study shows that IV Bu 3.2 mg/kg q day ×2 plus Flu 30 mg/m^2 q day ×5 is a well-tolerated, relatively intense regimen. It is desirable to control disease prior to transplantation, especially with reduced-intensity preparative regimens. A MUD transplantation is possible in older patients.

In summary, " It is a mistake to regard age as a downhill grade towards dissolution. The reverse is true. As one grows older, one climbs with surprising strides" — George Sand (1804-1876).

This research summary is presented as a brief guide to an important study that appeared in a recent issue of *Biology of Blood and Marrow Transplantation*. The complete paper, including all figures, tables, and references, can be found in Volume 11, Issue 5, Pages 354-361.

Long-term Immune Recovery of Patients Undergoing Allogeneic Stem Cell Transplantation: A Comparison with Their Respective Sibling Donors

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INTRODUCTION

Immunologic recovery after allogeneic stem cell transplantation (allo-SCT) plays an important role in the long-term outcome of patients after transplantation, because it influences the occurence of clinical complications that accout for most late posttransplantation mortality. The major goal of this study was to evaluate the status of the immune system at least 1 year after transplantation in a series of 38 patients who had received an HLA-identical sibling allo-SCT and to compare it with that of their respective related donors. Our interest was focused on the numeric and functional analysis not only of the different subsets of peripheral blood (PB) lymphocytes, but also of circulating dendritic cell (DC) subpopulations. The results were evaluated simultaneously in a patient/donor paired study performed only after complete bone marrow chimerism and normal PB cell counts were shown in all recipients. An additional goal of this study was to analyze the status of the immune system in a group of patients receiving a reduced-intensity conditioning (RIC) regimen as compared with those undergoing conventional myeloablative transplantations.

MATERIALS AND METHODS

Patients and Donors

From October 1995 to April 2000, 134 patients underwent a matched related donor allo-SCT in our institution. At the time the study was designed, 68 were alive and disease free. Of these, only 38 patients, together with their respective donors, finally gave their written informed consent to participate.

The median age was 42 years (range, 17-67 years) and 41 years (range, 17-73 years) in the patient and donor groups, respectively, whereas the male-female ratio was 20:18 in both groups. PB samples were simultaneously obtained from both patients and donors, and information on every parameter measured was available in all 38 pairs (there were no missing data).

RESULTS

Distribution of PB DCs

Although the total numbers of circulating DCs in patients who underwent sibling allo-SCT were not significantly different from those of their respective related donors (Table 2), interesting differences emerged with analysis of DC subsets. Thus, patients who had more CD16$^+$ DCs (P = .01), whereas both myeloid and plasmacytoid DC subsets were significantly decreased (P = .03 and P = .001, respectively) as compared with their respective sibling donors.

Distribution of PB NK, B-Cell, andT-Cell Subpopulations

Patients and their respective donors had similar numbers of circulating PB NK cells. As for DCs, a significant correlation was found between the number of NK cells in the patient and donor PB samples (P = .01; $r2$ = 0.4). This was the only clinical and transplant-associated parameter that influenced long-term NK cell numbers in the patient group. Conversely, at the time of this study, B-cell numbers were significantly higher in the patient group as compared with the donor group.

Regarding T cells, our results showed an inversion of the CD4$^+$/CD8$^+$ T-cell ratio that was due to both decreased CD4$^+$ T-cell numbers and increased CD8$^+$ T-lymphocyte counts,

Table 2. Distribution of Peripheral Blood Dendritic Cell Numbers in the Patient and Donor Groups

Parameter	Patients	Donors	P Value
Total DC count (×10^6/L)	59.0 ± 10	52.3 ± 6	NS
CD16$^+$ DCs (×10^6/L)	46.3 ± 10	33.7 ± 6	.01
Myeloid DCs (×10^6/L)	6.9 ±_1.4	8.9 ± 1.4	.03
Plasmacytoid DCs (×10^6/L)	6.2 ± 1.4	10.6 ± 2	.001

NS indicates not statistically significant; DCs, dendritic cells. Values are expressed as mean ± 2 SE.

in association with fewer double-positive $CD4^+/CD8^+$ T cells in the patient group.

Cytokine Production by Stimulated T Cells

T cells from patients displayed an increased production of TNF-α and IFN-γ together with a decreased production of IL-5, IL-2, and IL-10, as compared with their respective sibling donors.

Influence of Clinical Characteristics

Among the variables included in the general linear model analysis, the presence of chronic GVHD was the one with more influence on the immune system. $CD4^+$ T cells were found in this subgroup when compared to patients without this complication. The total number of T cells infused directly correlated with the absolute number of PB T cells ($P < .001$; $r2 = 0.639$) and with PB $CD4^+/CD8^{neg}$ ($P = .001$; $r2 = 0.54$) and $CD4^{neg}/CD8^+$ ($P = .02$; $r2 = 0.42$) T cells. Finally, there was no difference between patients undergoing RIC and those undergoing myeloablative transplantation in any of the immune system parameters analyzed (Table 5).

Table 5.Compared Immune System Status in Patients Receiving RIC versus Myeloablative Transplants

Parameter	RIC (n = 18)	Myeloablative (n = 20)
Total DCs ($\times10^6$/L)	62 ± 18	51 ± 12
$CD16^+$ DCs ($\times10^6$/L)	50 ± 16	37 ± 10
Myeloid DCs ($\times10^6$/L)	7.7 ± 1.6	6.6 ± 2.2
Plasmacytoid DCs ($\times10^6$/L)	5.7 ± 1.8	6.5 ± 2
NK cells ($\times10^6$/L)	269 ± 85	222 ± 74
B-cells ($\times10^6$/L)	466 ± 96	395 ± 55
Total T cells ($\times10^6$/L)	1637 ± 201	1431 ± 156
$CD4^+$ T cells ($\times10^6$/L)	774 ± 114	655 ± 74
$CD8^+$ Tcells ($\times10^6$/L)	767 ± 115	662 ± 95
$CD4^+/CD8^+$ T cells ($\times10^6$/L)	12 ± 4	13 ± 4
CD4neg/CD8neg T cells ($\times10^6$/L)	83 ± 24	99 ± 23
% TNF-α^+ cells $CD8^+$ cells	45 ± 10	44 ± 12
% TNF-α^+ cells $CD4^+$ cells	33 ± 10	37 ± 8
% IFN-γ^+ cells $CD8^+$ cells	66 ± 10	69 ± 8
% IFN-γ^+ cells $CD4^+$ cells	34 ± 6	38 ± 8
% $IL\text{-}4^+$ cells $CD8^+$ cells	0.9 ± 0.2	0.8 ± 0.2
% $IL\text{-}4^+$ cells $CD4^+$ cells	1.3 ± 0.4	1.3 ± 0.3
% $IL\text{-}5^+$ cells $CD8^+$ cells	2.1 ± 0.5	2.1 ± 0.4
% $IL\text{-}5^+$ cells $CD4^+$ cells	4.0 ± 1.4	4.4 ± 1.0
% $IL\text{-}2^+$ cells $CD8^+$ cells	12 ± 3	19 ± 4
% $IL\text{-}2^+$ cells $CD4^+$ cells	39 ± 6	45 ± 6
% $IL\text{-}10^+$ cells $CD8^+$ cells	0.5 ± 0.2	0.5 ± 0.2
% $IL\text{-}10^+$ cells $CD4^+$ cells	1.3 ± 0.4	1.2 ± 0.4

No significant differences were found between groups for any parameter measured.
Values are expressed as mean ± 2 SE.

DISCUSSION

Our results showed normal absolute DC and lymphocyte counts in the patient group compared with their donors at the time of study. Nevertheless, a more detailed analysis of these cells showed some differences between groups. Accordingly, patients displayed a different DC distribution compared with their donors, with an increased number of $CD16^+$ DCs and decreased counts of both myeloid and plasmacytoid DC subsets.

From the 3 major subsets of PB lymphocytes, B cells showed the most differences between patients and donors, with increased B cell numbers in the former group.

Regarding T cells, an inversion of the CD4/CD8 ratio was observed in our series. This was due to both a significant decrease in $CD4^+$ T cells and an increase in $CD8^+$ T lymphocytes. To the best of our knowledge this is the first study in which, in the long-term setting after allo-SCT, cytokine production by stimulated T-cell subsets has been specifically investigated at the cytoplasmic level on a single-cell basis. Our results indicated that, as compared with their respective donors, patients exhibited an increased production of TNF-α and IFN-γ associated with decreased numbers of IL-5– and IL-10–secreting T cells. These results, together with the increased $CD8^+$ T-cell numbers in PB, support the notion that at the study time point, a predominance of a T-helper (Th)1 T-cell response exists in patients undergoing allo-SCT in comparison to their respective donors.

In summary, our study shows the existence of several numeric and functional differences in distinct cellular compartments of the immune system of patients undergoing sibling allo-SCT, evaluated more than 1 year after transplantation, as compared with their respective donors. Nevertheless, because this was a descriptive study, the importance of the changes described in the posttransplantation setting should be further confirmed with larger series of patients and also by sequential analysis.

This research summary is presented as a brief guide to an important study that appeared in a recent issue of *Biology of Blood and Marrow Transplantation*. The complete paper, including all figures, tables, and references, can be found in Volume 11, Issue 8, Pages 576-586.

Effector Cells Derived from Host CD8 Memory T Cells Mediate Rapid Resistance against Minor Histocompatibility Antigen–Mismatched Allogeneic Marrow Grafts without Participation of Perforin, FasLigand, and the Simultaneous Inhibition of 3 Tumor Necrosis Factor Family Effector Pathways

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INTRODUCTION

Bone marrow transplantation (BMT) is an important therapeutic modality for the treatment of many hematopoietic malignancies and other disorders. Graft failure due to immunologic resistance against donor allogeneic bone marrow (BM) grafts by the recipient is a major concern in clinical transplantation. The contribution of individual cellular subsets can differ depending on

transplantation parameters such as the genetic disparity between donor and recipient and the status of host antidonor reactivity.

We and others have previously studied the contribution of cytotoxic pathways to T cell–mediated resistance against donor progenitor/stem cells in a donor alloantigen–sensitized experimental model. Donor antigen–sensitized recipient mice deficient in both perforin and Fas ligand (FasL) function (B6-cdd) maintain strong resistance to major histocompatibility complex (MHC)–mismatched (BALB/c: H2d) and MHC-matched/minor histocompatibility antigen (MiHA)–mismatched (C3H.SW or BALB.B: H2b) BM.

This investigation examined the early kinetics of resistance in donor antigen–sensitized B6-cdd and wild-type B6 (B6-wt) recipients after transplantation of MiHA-disparate BM cells (BMCs). By using BALB.B donors in this study, antigen-specific CD8 memory T cells (T_M) cells against the immunodominant H60 minor transplantation antigen were identified in recipients before transplantation. Findings demonstrate that resistance against progenitor cells in these recipients is an effective and rapidly occurring event mediated by host CD8 T cells whether or not the major cytotoxic pathways are functional in transplant recipients (Figure 1). We further investigated the operative molecular pathway of this T_M-dependent resistance. Involvement of 2 additional TNF family ligands capable of inducing apoptosis has not been previously examined in allograft resistance. Tumor necrosis factor–like weak inducer of apoptosis (TWEAK) and TL1A have both been shown to be expressed on activated T cells. TWEAK has been shown to be capable of weakly inducing apoptosis upon binding its receptor Fn14, whereas TL1A binds DR3, 1 of a family of receptors that contains a cytoplasmic cysteine-rich protein interaction motif termed the *death domain*, which is capable of recruitment and activation of procaspase 8 and initiation of cell death. Together with deficiencies in perforin and FasL, recently produced monoclonal antibodies (mAbs) were used and shown to block apoptotic signaling through Fn14, DR3, and DR5 individually and in combination to disrupt 3 additional cytotoxic pathways in transplantation models. The simultaneous disruption of 5 signaling pathways did not alter the strong resistance mediated by cytotoxically defective antigen-sensitized recipient mice (Figure 5). Together with our previous findings, these

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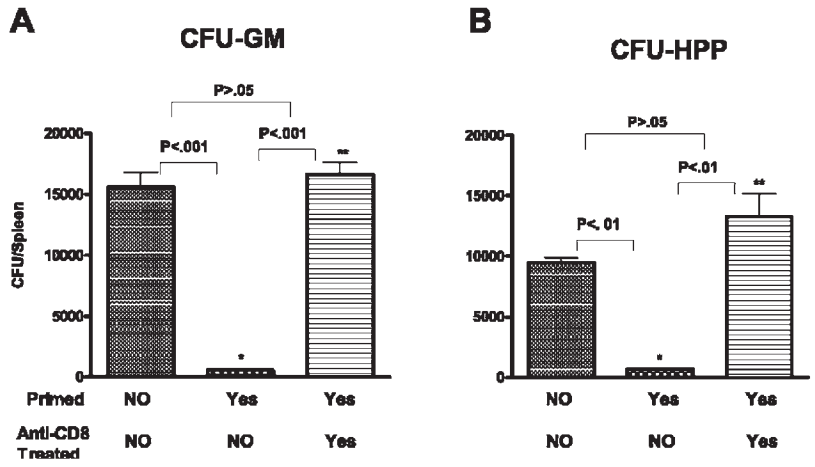

Figure 1. B6-wt (H2b) recipients were sensitized to C3H.SW (H2b) alloantigens and administered anti-CD8 mAb (see "Materials and Methods") to deplete host $CD8^+$ T cells before transplantation. Controls were treated with phosphate-buffered saline. After 9.0 Gy of total body irradiation, B6-wt recipients underwent transplantation with 1 × 107 C3H.SW TCD BM cells. Unsensitized recipients also underwent transplantation as controls. At 5 days after transplantation, recipient splenocytes were harvested and assayed for CFU-granulocyte macrophage (CFU-GM) (A) or CFU-HPP (B). Data represent CFUs per spleen (mean ± SEM). Statistical differences between groups were determined by analysis of variance with the Tukey multiple comparison test.

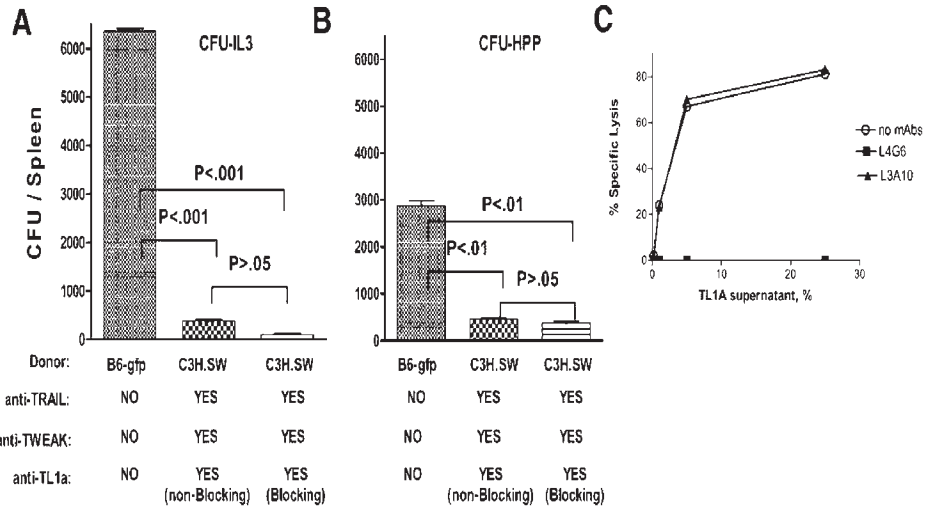

Figure 5. A and B, B6-cdd mice were sensitized to donor alloantigens (C3H.SW) and then underwent transplantation with 1 × 107 TCD BM cells from allogeneic donors (C3H.SW) or B6-gfp as a syngeneic control. On days –1 and 0 before transplantation, recipients received no antibody, 250 μg of anti-TRAIL, 250 μg of both anti-TRAIL and anti-TWEAK, or 250 μg each of anti-TRAIL, anti-TWEAK, and anti-TL1A (L4G6) blocking mAbs. On day 5 after transplantation, recipient splenocytes were harvested and cultured for the presence of CFU–IL-3 (A) or CFU-HPP (B). Data represent the number of CFU s (mean ± SD; n = 3-4 per group). C, TL1A-containing supernatant was tested against 51Cr-labeled EL4-DR3 and was shown to effect lysis in a 4-hour release assay. Varying amounts of TL1A supernatant were then mixed with L4G6-and L3A10-containing supernatants in a 1:1 volume ratio and preincubated for 30 minutes before addition to these target cells. Data represent mean specific lysis from triplicate cultures.

New Era of Preparative Regimens: Controlled Ablation and Reduced Toxicity

CME Assessment Test

1. **Which of the following is true concerning either myeloablative or nonmyeloablative therapy prior to HSCT?**
   A. Full myeloablation is no longer a necessary goal in HSCT, regardless of disease or type of transplant.
   B. Accurate dosimetry of either chemotherapy or radiotherapy will allow therapy to increase engraftment and potentially decrease both acute and long term toxicities.
   C. Immunosuppression is no longer required as part of the preconditioning therapy.
   D. A single approach for reducing graft versus host disease has been defined and is commonly used in both nonablative and myeloablative transplant approaches.
2. **Which of the following is true regarding minimal intensity conditioning regimens prior to transplantation?**
   A. Use of postgrafting immunosuppression to control graft rejection and GVHD reduced toxicities associated with allografting.
   B. Seattle dog studies showed the abilities of TBI, cyclosporine and MMF to promote engraftment in the nonmyeloablative transplant environment.
   C. Using various doses of busulfan and TBI in congenic and MHC-mismatched and mismatched transplanted mice, TBI was found to be more immunosuppressive than busulfan promoting sustained donor cell engraftment when combined with cyclophosphamide.
   D. All of the above.
3. **In a recent prospective clinical study which one of the following findings is false?**
   A. The use of IV busulfan is associated with less regimen related toxicities in both related and unrelated stem cell transplantation.
   B. The fludarabine/IV busulfan regimen has a better toxicity profile as compared with fludarabine/melphalan regimen.
   C. Fludarabine/IV busulfan × 4 days results in better disease control compared to fludarabine IV busulfan × 2 days in leukemia patients with active disease.
   D. None of the above.
4. **Which of the following is false?**
   A. Once daily IV busulfan fludarabine is a safer posttransplant conditioning regimen than the IV busulfan/cyclophosphamide combination for allogeneic HCT.
   B. An average daily AUC of approximately 6,500 μMol-min over a 4-day treatment course improves treatment for patients transplanted with high-risk disease.
   C. PK guidance to deliver individualized myeloablative conditioning therapy is safe but labor intensive.
   D. None of the above.
5. **In selecting optimal conditioning regimens, which one of the following does not have to be considered?**
   A. Patients age and donor source
   B. Patients general medical condition
   C. Recipient immune competence
   D. None of the above.

CME Evaluation Form

Please evaluate the effectiveness of this CME activity on a scale of 1 to 5, with 5 being the highest, by circling your choice. Fax *with the Answer Sheet* to the Office of Continuing and Professional Education, 414-456-6623, or mail to the Office of Continuing Education, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226.

Overall Quality of the CME Activity	1	2	3	4	5
Articles in the publication were presented in a clear and effective manner.	1	2	3	4	5
The material presented was current and clinically relevant.	1	2	3	4	5
Educational objectives were achieved.	1	2	3	4	5
The CME activity provided a balanced, scientifically rigorous presentation of therapeutic options related to the topic, without commercial bias.	1	2	3	4	5

Please comment on the impact (if any) that this CME activity might have on your management of patients.

Would you benefit from additional CME programs on this topic? Yes No

I have read these articles on the new era preparative regimens, published in *Blood and Marrow Transplantation Reviews*, and have answered the CME test questions and completed the Evaluation Form for this activity.

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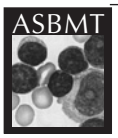
CME Assessment Test Answer Sheet

Release Date: October 31, 2005 Last Review Date: October 31, 2005 Expiration Date: October 31, 2006

Instructions

(1) Read the articles in the publication carefully. (2) Circle the correct response to each question on the Answer Sheet. (3) Complete the evaluation Form. (4) To receive CME credit, fax the completed Answer Sheet and Evaluation Form to the office of Continuing and Professional Education (414-456-6623) or mail to the Office of Continuing Education, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226. No processing fee is required.

1.	A	B	C	D	**3.**	A	B	C	D	**5.**	A	B	C	D
2.	A	B	C	D	**4.**	A	B	C	D					



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observations are consistent with the notion that if the killing of donor progenitor cells occurs during resistance responses, at least 1 cytotoxic pathway distinct from TWEAK, TL1A, TRAIL, FasL, perforin, and TNF is operative and effective in mediating strong resistance by CD8 T_M against MiHA-mismatched BM progenitor/stem cell allografts. These findings support further investigation to address the precise role of apoptosis and cellular cytotoxicity in allogeneic stem/progenitor cell rejection.

DISCUSSION

This study extends previous observations from our laboratory and others concerning resistance to marrow allografts in the absence of perforin and FasL by comparing their early T-cell responses with that in B6-wt mice and examining resistance while simultaneously inhibiting these additional cytotoxic ligands. The findings indicate that a CD8 T cell–mediated effector modality independent of perforin and the TNF family ligands FasL, TRAIL, TWEAK, and TL1A can rapidly mediate resistance against allogeneic hematopoietic stem/progenitor grafts in recipients sensitized against donor MiHA allogeneic antigens.

An important observation in this study was the finding that rapid resistance assessed by the presence/absence of donor progenitor cells (ie, within 48 hours) was detected in both cytotoxically normal and perforin and FasL cytotoxically deficient recipients. The presence of T_M in sensitized mice is consistent with the rapid and efficient resistance detected. We interpret the results to indicate either that the same effector pathways are being used in both cytotoxically normal and cytotoxically deficient mice to mediate resistance or that different pathways with virtually identical kinetics are being used in these 2 groups of recipient mice. In either case, the findings demonstrate that CD8 T_M can mediate potent resistance against allogeneic progenitor cell populations through a mechanism that does not include the major cytolytic perforin/granzyme- and FasL-mediated pathways.

In summary, our findings may be interpreted in at least 2 ways. First, resistance in recipients sensitized against donor antigens may be a result of a nonperforin/FasL-mediated cytotoxic effector pathway that is presently undefined. Alternatively or in addition, the involvement of noncytotoxic resistance pathways—for example, mechanisms that block the maturation or differentiation of progenitor cells—could also be important. Understanding the pathways of resistance mediated by effector cells derived from naive T cells and T_M after both ablative and nonmyeloablative conditioning regimens will be important for the development of effective strategies for support of allogeneic progenitor cell engraftment and tolerance induction after hematopoietic stem cell transplantation.

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