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REVIEWS

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IN THIS ISSUE

Introduction

Antithymocyte Globulin: Teaching Old Dogs New Tricks

by John R. Wingard, MD, Editor

Antithymocyte globulin (ATG) has been used in hematopoietic stem cell transplantation (HSCT) for several decades. Its important role in the treatment of aplastic anemia was noted early, and its usefulness has remained through the decades both as part of the conditioning regimen for some HSCT applications and as a therapy for acute graft-versus-host disease (GVHD). Multiple polyclonal and monoclonal antibody preparations have been developed over the years. There are differences in these various products, but the clinical significance of these differences is uncertain. Comparative trials have not been conducted, and today it remains unclear as to whether one product might be more suited for a specific application than another. Moreover, comparative trials have been made difficult by the lack of clear convertibility of different doses and dose schedules of the various products.

There are important differences between the anti–T-cell antibody products and pharmacologic agents that suppress T-cell function. The prolonged presence of antibody in the circulation and its ongoing activity in vivo for days to weeks is a clear-cut difference. This activity could have a beneficial effect by dampening deleterious immunopathologic reactions. In some situations, however, this prolonged action can have disadvantageous effects such as increasing susceptibility to infection or lymphomas.

These proceedings of a symposium held at the 2002 Tandem BMT Meetings present new data from pilot studies to evaluate the role of ATG in new HSCT applications. Dr. Nash discusses ATG use in the conditioning regimen of patients undergoing high-dose immunosuppressive (not cytotoxic) therapy prior to stem cell infusions as therapy for immunologic disorders. Dr. Blazar presents a compilation of data from the University of Minnesota regarding the use of ATG as therapy for patients with refractory acute GVHD and examines factors that influence outcome. Dr. Spitzer considers the role of ATG in nonablative transplantation conditioning regimens. Finally, Dr. Laughlin reports outcome data from patients undergoing cord blood transplantation in which ATG was incorporated into the conditioning regimen.

These presentations have the unifying theme of examining the utility of a time-tested biological preparation for new HSCT applications. These data suggest that ATG will continue to have an important role in HSCT decades later. Is horse ATG a better agent for suppression of T-cell function than other biologic preparations or pharmacologic agents developed to accomplish the same purpose? Unfortunately, these datasets do not answer this question, which must be addressed using controlled trials, larger sample sizes, and different study designs to probe the individual contribution of ATG. Notwithstanding, these data are helpful in setting the stage for consideration of such issues in subsequent studies and in clarifying both the benefits and limitations of these agents in current HSCT practice.

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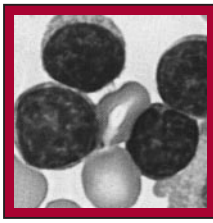

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ASBMT News

2003 Tandem BMT Meetings Will Be Jan. 30 - Feb. 3 in Keystone, Colorado

The combined annual meetings of ASBMT and the International Bone Marrow Transplant Registry/Autologous Blood and Marrow Transplant Registry (IBMTR/ABMTR) will be Jan. 30 - Feb. 3 at Keystone Resort in Colorado.

In addition to the five days of scientific sessions for BMT clinicians and investigators, there will be five related conferences and courses.

- Clinical Research Associates Data Management Workshops: Jan. 29-Jan. 31
- BMT Center Medical Directors Conference: Feb. 2
- Oncology Nursing Society Conference: Feb. 1-3
- BMT Pharmacists Conference: Jan. 29-30
- BMT Center Administrators Conference: Jan. 31-Feb. 1

Details about registration and conference programs can be found on the ASBMT Web site at ***www.asbmt.org***.

Meeting Registration

The early registration deadline is **October 21, 2002**. Register online at the ASBMT or IBMTR/ABMTR Web site.

Abstract Submission

The deadline for abstracts is **October 21, 2002**. Abstracts may be submitted online at the ASBMT or IBMTR/ABMTR Web site.

Housing Accommodations

The housing deadline is **December 13, 2002**. Download and print the Housing Reservation Form from the ASBMT or IBMTR/ABMTR Web site. Or, call Keystone Resort reservations toll-free at (800) 222-0188 and mention the Tandem BMT Meetings to obtain special conference rates.

Scientific Topics

Recent advances in the broad fields of cellular therapy and blood and marrow transplantation will be addressed in plenary sessions, concurrent sessions, workshops, poster sessions and symposia. Topics will include:

- Antigen-Specific Immune Reconstitution
- Biology of GvHD
- Clinical Trial Design
- Dendritic Cells
- Detection of Antigen-Specific Cells
- Drug Interactions
- Graft-versus-Tumor Therapies
- Homing
- Immunogenetics of BMT
- Natural Killer Cells
- Non-Malignant Pediatric Disorders
- Novel Autotransplant Regimens
- Novel Immune Suppressive Therapies
- Post-Transplant Lung Disease
- Quality of Life
- Stem Cells for Tissue Regeneration

Keystone Resort

High in the Colorado Rockies, Keystone Resort offers first-class accommodations in a spectacular winter sports setting and a revitalizing atmosphere in which to work and unwind. Conference participants can choose sleeping accommodations from more than 1,400 condominiums, studio apartments and hotel rooms. Additional resort information is at ***www.keystone.snow.com***.

Preliminary Scientific Program Announced for Tandem BMT Meetings

The organizing committee for the 2003 Tandem BMT Meetings, chaired by **Nelson Chao, MD**, for ASBMT and **Olle Ringdén, MD**, and **Richard Champlin, MD**, for IBMTR/ABMTR, has assembled a scientific program of basic and clinical science that will include 35 to 40 speaker presentations based on submitted abstracts.

Major sessions and workshops at the Jan. 30-Feb. 3 conference in Keystone include:

Thursday, January 30

- Immunogenetics of Bone Marrow Transplantation–*Bo Dupont, John Hansen, Warren Schlomchik*
- NK Cells–*Judith Ann Shizuru, Peter Parham, Andrea Velardi, William Murphy*
- Drug Interactions–*Michael Colvin, William Plunkett*
- Oral Abstracts

Friday, January 31

- Antigen-Specific Immune Reconstitution –*Robertson Parkman, George Hollander, Janice (Wes) Brown*
- Dendritic Cells–*James Mule, James Young, David Munn, Katharine Whartenby*
- BMT for Pediatric Immune/Inflammatory Disorders–*Alexandra Filipovich, Jean Laurent Casanova, Nico Wulffraat*
- Detection and Characterization of Antigen-Specific Cells–*Jeffrey Molldrem*
- Lung Injury After SCT: How Can We Improve Early Diagnosis and Treatment? –*Kenneth Cooke, Joan Clark*
- Clinical Trials: Statistical Questions and Answers–*John Klein, Glenn Heller*
- Poster Session I

Saturday, February 1

- Novel Regimens for Autologous Transplantation–*Julie Vose, Richard Jones, Robert Negrin*
- Donnall Thomas Lecture–*To Be Announced*
- Oral Abstracts
- Best Abstracts

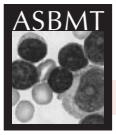
Sunday, February 2

- Homing–*Richard O'Reilly*
- Transplant Center Resources–*Mary Horowitz, Fausto Loberiza, Jane Apperley, Richard Watt*
- Novel Immune Suppressive Therapies– *Bruce Blazar, Randoph Noelle, Claudio Anasetti, Samuel Strober*
- PBSC from Unrelated Donors: Can We Exploit Them to Improve Outcome?– *Claudio Anasetti, Dennis Confer*
- Graft-versus-Tumor Strategies: Can We Separate GvT from GvHD?"–*Richard Champlin, Frederick Falkenburg*
- Quality of Life: How to Assess It?– *Stephanie Lee, Gérard Socié*
- Poster Session II

Monday, February 3

- GvHD Biology–*James Ferarra, Robert Korngold, Tuna Mutis*
- Non-Myeloablative Transplantation (International Session)–*Alexandra Filipovich, Jane Apperley, Alois Gratwohl, Charles Crawley, Christopher Bredeson*
- Using Registry Data to Answer Clinical Questions–*Olle Ringdén, Daniel Weisdorf*
- Critical Clinical Trials–*John Wingard, Jane Apperly*
- Oral Abstracts
- Stem Cells for Tissue Regeneration– *Armand Keating*

Members of the ASBMT Annual Meeting Committee include **Mitchell Cairo, MD**, **James L. M. Ferrara, MD**, **Helen Heslop, MD**, and **Judith Shizuru, MD**.



SYMPOSIUM Report

Adapted from a symposium sponsored by an unrestricted educational grant from Pharmacia.

The Evolving Role of Equine Antithymocyte Globulin in Hematopoietic Stem Cell Transplantation

2002 Tandem BMT Meeting

February 26, 2002, Orlando, Florida

Richard A. Nash,[a] Bruce R. Blazar,[b] Thomas Spitzer,[c] Mary J. Laughlin[d]

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Richard A. Nash, MD
High-Dose Immunosuppression in the Autoimmune Transplantation Setting: Lessons Learned

In preparation for randomized studies that are being planned through the National Institutes of Health (NIH), 2 pilot studies of high-dose immunosuppression treatment (HDIT) and autologous stem cell transplantation (SCT) for severe autoimmune diseases have been done by the Seattle Consortium. The studies were multicenter and required the collaboration of stem cell transplantation physicians, neurologists and rheumatologists. The objective of the pilot studies was to determine safety of HDIT and SCT in patients with severe multiple sclerosis (MS) and systemic sclerosis (SSc). In both MS and SSc patients, the primary goal of therapy was to prevent disease progression.

Treatment

An outline of treatment is presented in Table 1. Granulocyte colony-stimulating factor (G-CSF) at a dosage of 16 µg/kg per day was administered to all patients for mobilization. Prednisone was administered to the patients with MS after the fourth patient had an MS flare while receiving G-CSF during mobilization. That flare occurred approximately 24 hours after the initiation of G-CSF therapy. A report by Openshaw et al [1] described 4 patients who had flares while receiving G-CSF. After the addition of prednisone to the mobilization regimen used for the MS patients only, no further flares occurred. A minimum of 3.5×10^6 $CD34^+$ cells/kg was collected, plus backup cells. The HDIT regimen consisted of total body irradiation administered on day –5 and day –4, cyclophosphamide on day –3 and day –2, and then equine antithymocyte globulin (ATG) (Atgam; Pharmacia, Peapack, NJ) to a total dose of 90 mg/kg administered from day –5 to day +5. Lung shielding to a total organ dose of 200 cGy was done for the SSc patients only. The adminstration of horse ATG was continued after autologous hematopoietic stem cell transplantation (HSCT) in an attempt to in vivo deplete T-cells that were in the graft. After transplantation, patients received G-CSF until they recovered their counts. Prednisone was administered after transplantation. The patients on the SSc pilot study received prednisone to prevent further inflammatory changes in the lungs associated with the administration of the intensive immunosuppressive regimen. This prophylaxis was thought to be necessary because most of the patients entered into the HDIT protocol for SSc had pulmonary disease with a carbon monoxide diffusing capacity (DLCO) as low as 45% or 50%. Prednisone after HDIT was added to the routine supportive care of all MS patients to prevent the development of the engraftment syndrome, which was associated with a mostly transient but significant increase in weakness. In patients with SSc, 2 of the first 8 patients developed idiopathic pneumonia syndrome. After patient no. 8, the lungs were shielded to prevent this complication. $CD34^+$ cells were collected from patients for a total of 3 days. The median number of $CD34^+$ cells infused was 4.6×10^6/kg. The average purity was 84%, somewhat lower than expected. Purities of the autologous graft were improved with the use of the newer software for the Isolex 300i (Baxter Healthcare, Deerfield, IL). Engraftment was very quick, occurring in all patients by a median of day 9 for both neutrophils and platelets.

HDIT and Autologous HSCT for MS

A total of 26 patients entered the first MS study, which is now closed. The results of this study are summarized in Table 2. The median age of the patients was 41 years. Eighteen of the patients had secondary progressive disease, 7 patients had

Table 1. Treatment Schema

Mobilization		
	G-CSF	
	Prednisone 1 mg/kg per day for 10 days starting 1 day before G-CSF (MS patients only)	
HDIT		
	Day	
	–5	TBI* 200 cGy (×2); horse ATG 15 mg/kg per dose
	–4	TBI* 200 cGy (×2)
	–3	Cyclophosphamide 60 mg/kg; ATG 15 mg/kg
	–2	Cyclophosphamide 60 mg/kg
	–1	ATG 15 mg/kg
	0	Stem cell infusion
	+1	ATG 15 mg/kg
	+3	ATG 15 mg/kg
	+5	ATG 15 mg/kg
Posttransplantation		
	G-CSF from day 0 until ANC > 500	
	Prednisone 0.5 mg/kg/day from day 7 to 21 (MS patients only)	
	Prednisone 0.5 mg/kg/day from day –6 to 30 and tapered (SSc patients only)	

**Lungs are shielded for SSc patients.*

primary progressive disease, and 1 patient had relapsing remitting disease. All patients except those with primary progressive disease had failed conventional therapy. There is no US Food and Drug Administration–approved therapy for primary progressive disease, so those patients could be admitted onto protocol directly if they met the inclusion criteria. Six of the patients had enhancing lesions on gadolinium infusion at baseline. The median expanded disability status scale (EDSS) score, a standard measure of neurologic dysfunction associated with MS, was 7. For this report, the median patient follow-up was 12 months. Follow-up evaluations were performed at 1, 3, 6, 12, and 24 months, so there was a big gap in evaluations between the 12- and 24-month periods.

Two neurotoxic events occurred. One patient, who has already been mentioned, developed an MS flare with transient paraparesis during mobilization with G-CSF. Her EDSS score increased from 6.5 to 7.5, and results of magnetic resonance imaging (MRI) 1 month after transplantation were positive for a new enhancing lesion. At approximately 3 months after transplantation, this patient completely recovered the neurologic function that she had lost as a result of the G-CSF mobilization.

The second event, which was somewhat more concerning, occurred in a patient who developed fever after transplantation at approximately at 10 days, right around the time of engraftment. This patient developed paraparesis, and his EDSS score went from 7.5 to 8.5. MRI results of the brain and spinal cord were negative. After this event occurred, all of the patients were reassessed, and it was noted that 13 of the 18 patients on this protocol had some degree of fever, usually low grade, or inflammatory changes in the skin or lungs. Because many patients will decompensate neurologically when they develop fever, we admitted patients to the hospital if a fever developed. After we determined that a high number of patients were developing fevers, prednisone was added to the regimen in an attempt to control the development of fever along with inflammatory symptoms and signs. After addition of prednisone for the subsequent 8 patients, no further problems were noted, but certainly further evaluation is needed.

One patient in this protocol developed Epstein-Barr virus (EBV) lymphoma, and 1 patient developed Guillain-Barré syndrome at 18 months. It is unclear what the relationship of the Guillain-Barré syndrome is to the regimen, but we mention it here because there may be a relationship between the transplantation and the development of Guillain-Barré syndrome. Infections were not a significant problem. Nine patients were seropositive for cytomegalovirus (CMV). CMV infection reactivated in 4 of these patients after transplantation, and the 1 patient who developed CMV disease was the patient who later developed the EBV lymphoma.

As previously stated, the goal of the treatment was to prevent further progression of neurological dysfunction. As of this report, 5 of the 26 patients have had further progression, defined as an increase in the EDSS score by at least 1 point. Including the patient who developed a new lesion associated with mobilization, there were 3 patients with new or enhancing lesions at 1 year after high-dose therapy. Cerebrospinal fluid (CSF) analysis for the presence of oligoclonal bands, which are considered a marker for the inflammatory process, was performed on patients at baseline and follow-up. Although the CSF from some patients became negative, HDIT did not seem to have a significant effect on the presence of oligoclonal bands after treatment.

HDIT and Autologous HSCT for SSc

To date, 27 patients have been included in the SSc study. The results are summarized in Table 3. A report on the first 19 patients, who have been fully analyzed for outcome as well as side effects, has been submitted for publication (P. McSweeny, unpublished observations), and this presentation focuses predominantly on these 19 patients. The other 8 patients were put on study fairly recently. Eighteen of these 19 patients had lung disease as the primary reason that they were undergoing transplantation. Ten of these patients were positive for the autoantibody SCL70, which is a marker for SSc. The median age of the patients was 39 years, and the median duration of the disease prior to transplantation was 20 years. Results of analysis of baseline status at the time of transplantation indicated a median modified Rodman skin score of 31. The scale of the Rodman skin score is from 0 through 51, with 51 indicating the maximum disease severity. Patients are considered to have extensive disease if they have a skin score greater than 16, so these patients had fairly extensive disease. The median DLCO in terms of percent predicted was 57%; forced expiratory capacity was 72%, and the renal function was actually quite good in this patient population, with a median of 0.7 mg/dL.

Complications and problems seen in this group included development of idiopathic pneumonia syndrome in 2 of the first 8 patients. Because of this complication, lung shielding during radiation treatment down to a total dose of irradiation of 200 cGy was initiated, and no further pulmonary complications occurred in the subsequent 17 patients nor in the more recently treated 8 patients.

One patient in this protocol developed EBV posttransplantation lymphoprolifera-

Table 2. HDIT and Autologous HSCT for MS

Toxicities were identified and the protocol amended.
Prednisone was added for mobilization.
Other forms of ATG prohibited.
Prednisone added after HDIT/SCT to prevent fever.
Other toxicities transient and reversible.
Mortality was 4% (1/26) but may be lower in the future.
Five patients (5/26) had either or both clinical and MRI progression after HDIT/SCT.
Randomized NIH-sponsored study is planned.

Table 3. HDIT and Autologous HSCT for SSc

Responses in skin disease have been observed in advanced severe SSc.
Skin responses were associated with major improvements in quality of life (HAQ).
Internal organ functions stable overall. Transient decrements in lung function (DLCO) with TBI (8 Gy)/Cy/ATG.
Fatal lung injury in 2 patients. No other episodes after lung shielding during TBI was initiated. Transplantation-related mortality after shielding was 1/19 (5%).
SSc remains challenge for the application of HDIT. NIH-sponsored study is planned.

tive disease (PTLD), which is discussed below. One patient had problems with disease progression at approximately 3 to 4 months after transplantation and had a renal crisis. Another patient, within a month of this report, also suffered renal failure necessitating dialysis. It is not clear at this time if that episode was related to a renal crisis associated with the SSc or if it was related to acute tubular necrosis. Recently, a patient returned who was diagnosed with myelodysplastic syndrome (MDS), and this patient was cytogenetically positive for monosomia 7.

As in the MS patient group, infections were not much of a problem in this patient group. Thirteen patients were seropositive for CMV; 4 of these patients had CMV reactivation, and no patient developed CMV disease. One patient developed a problem with recurrent resistant herpes simplex virus (HSV); this patient had developed EBV lymphoma.

The results in the SSc study have been fairly satisfactory in that the goal of these studies was to prevent disease progression, but a significant improvement has occurred in one of the organs involved with SSc, the skin. There has been a significant reduction in the Rodman skin score after HDIT compared to the baseline, indicating a positive response to treatment. In this group of 19 patients, with a minimum follow-up of 12 months, there was a reduction in the skin score of at least 13, with a highly positive *P* value. The percent Rodman skin score after transplantation compared to baseline also showed a downward trend for virtually all the patients over the period of time that they were followed after transplantation.

There was also a significant improvement in function as measured by the modified Health Assessment Questionnaire (M-HAQ), a functional score with a scale from 0 to 3, with a score of 3 indicating severe disability. The overall M-HAQ score showed a reduction of 1.7, again with a very significant *P* value. In most of the patients, we saw a fairly dramatic reduction in the M-HAQ or improvement in function that we believe was related to the improvement in the skin score. These patients also showed a slight increase in forced vital capacity and some initial reduction in DLCO, with a nonsignificant *P* value, followed by stabilization.

Combined MS and SSc Study Results for Evaluation of EBV Lymphoma

EBV lymphoma developed in 2 patients after transplantation, 1 patient in each of the 2 protocols. These 2 patients were basically treated back-to-back, with the rabbit ATG administration occurring virtually within weeks of each other. The first patient was the patient with SSc, who was antinuclear antibody (ANA) positive and antitopoisomerase I (Scl70) negative. This patient had a positive skin test to horse ATG. We treated with a full 6-dose course of rabbit ATG (total dose 15 mg/kg). Early after transplantation, the patient developed acyclovir-resistant HSV stomatitis, which led to hospital readmission. This infection was treated and was resolving. Then on day 48, the patient developed bilateral lung infiltrates and was intubated. Later on, EBV lymphoma was diagnosed, and this diagnosis was confirmed on autopsy.

The second patient had MS and also a positive skin test to horse ATG. This patient also received a full course of rabbit ATG at the same dose as the first patient. CMV pneumonitis developed on day 14 as well as gastroenteritis. The patient was treated with double-agent therapy, ganciclovir and cidofovir with CMV immunoglobulin (Ig). On day 49, bilateral lung infiltrates were seen, similar to what was observed in the previously described patient. Although a definitive diagnostic procedure was completed fairly quickly and treatment started, the patient died within 4 days with disseminated disease.

The combined MS and SSc studies include a total of 53 patients to date. At the time that the studies included 51 patients, 46 patients received horse ATG only. Two patients had received a full course of rabbit ATG only, and those were the patients that developed EBV lymphoma. Two patients got a partial course of rabbit ATG after horse ATG was discontinued because of infusion reactions. The 2 patients who received only a partial course of rabbit ATG did not have significant posttransplantion infections or develop EBV lymphoma. One patient received no horse ATG or rabbit ATG.

T-cell analysis on day 28 showed no $CD4^+$ or $CD8^+$ cells evident in the peripheral blood of the 4 patients who either received a partial or full course of rabbit ATG. The day 28 CD4 and CD8 T-cell–count values in the peripheral blood of these 4 patients were significantly less than those in patients who received horse ATG only. This finding suggests that the degree of immunosuppression induced in these patients has to be limited to avoid the risk of EBV lymphoma (Table 4).

Summary

To summarize our experience to date, this pilot study investigated HDIT in a population of patients for whom there was very limited data on the use of HDIT in autologous HSCT. As a result of experience gained in this study, we added prednisone to the mobilization regimen for patients with MS and are now shielding the lungs for patients with SSc. We have prohibited other forms of immunosuppressive therapy and other forms of ATG for both patient populations. We have added prednisone after HDIT and transplantation for MS patients. Most of the toxicities that occurred early after transplantation were transient and reversible, and patients did not have problems with infection. The MDS incidence is yet to be determined with longer follow-up, although MDS development is potentially a problem. The

Table 4. Limits to the Intensity of Immunosuppression for HDIT and Autologous HSCT

Standard ATG in HDIT is horse ATG. However, 4/53 patients received rabbit ATG.
Two patients treated with rabbit ATG (15 mg/kg) developed EBV-PTLD.
In patients treated with rabbit ATG, CD4 and CD8 T-cell counts were 0/mmL on day 28.
EBV-PTLD is a rare complication after autologous HSCT but may necessitate limiting the intensity of HDIT.

mortality rates were 1 of 26 patients for MS and 4 of 27 for SSc; 3 of the SSc patients died of transplantation-related complications. Therapeutic responses were observed in SSc patients. Treatment efficacy is yet unclear in MS because of the lack of good historical controls to determine whether disease progression has been prevented in this patient population. Further follow-up is clearly needed to assess the durability of response, and randomized studies with the NIH are currently planned. Peter McSweeny will be leading one of the other studies, and there are 3 transplantation physicians involved with the MS study, Chris Bredison, Richard Burt, and Richard Nash.

Questions

Participant. What are we going to be doing for patients who have positive skin tests to horse ATG?

Dr. Nash. Currently, because of the experience in these studies and the uncertainty as to what degree of ATG can be added to the regimen, patients are being treated only with cyclophosphamide and total body irradiation (TBI) and not with ATG.

Participant. When you did your oligoclonal bands, did you use spinal fluid analysis to measure any of the albumin–IgG blood-brain barrier changes that can occur to see if there was any change post-BMT in the MS patients?

Dr. Nash. No. We have not done that. We have basically just been following the oligoclonal bands. In terms of the blood-brain barrier and determining if there were still problems with respect to an inflammatory process, we have been doing serial MRIs with gadolinium infusions at 1, 3, 6, 12, and 24 months.

Participant. The conditioning regimen that you are using is not stem cell toxic. Do you indeed need stem cell support or could you do the same thing with just supportive growth factors?

Dr. Nash. That is a good question. Some of the regimens currently being used for treatment of severe autoimmune diseases are those with high-dose cyclophosphamide. I think it is clear that patients on those regimens will recover their counts without stem cell support. With the regimen we are using, TBI-800 with cyclophosphamide, I don't think anybody is certain that it's myeloablative, but certainly patients would have a very prolonged period of neutropenia so that yes, stem cell support is required.

Reference

1. Openshaw H, Stuve O, Antel JP, et al. Multiple sclerosis flares associated with recombinant granulocyte colony-stimulating factor. *Neurology.* 2000;54:2147-2150.

Bruce R. Blazar, MD

The Early Use of Equine ATG in Nonmyeloablative Stem Cell Transplantation

In a study conducted between August 1990 and November 1998, a series of patients were treated at the University of Minnesota with equine antithymocyte globulin (ATG) for steroid-resistant acute graft-versus-host disease (GVHD). The first goal of this study was to determine the efficacy and long-term outcome in patients receiving equine ATG as first-line therapy for treating steroid-resistant acute GVHD in the 1990s at a single institution. The second goal was to determine the relative efficacy and long-term outcome in patients who received hematopoietic stem cell transplants (HSCT) from related donors (33 patients) and in patients who received HSCT from unrelated donors (45 patients) and also received equine ATG for steroid-resistant acute GVHD. A report of this study was recently published in *Biology of Blood and Marrow Transplantation* [1].

Acute GVHD Diagnosis and Grading

For diagnosis and grading systems, we used the clinical grading system developed by the GVHD Consensus Conference as well as the International Bone Marrow Transplant Registry (IBMTR) Severity Index, which includes upper gastrointestinal (GI) GVHD. Diagnoses were confirmed histologically whenever possible. The initial score was the maximum stage in each organ during a 15-day window from day –10 to day +5, with day 0 being the day of initiation of ATG therapy. The grade was determined by computer algorithm with all available clinical GVHD organ-scoring data. Grading was centrally reviewed by either Dr. Dan Weisdorf or Dr. Stella Davies, and it was prospectively recorded in the University of Minnesota Hospital BMT data base.

Initial Acute GVHD Therapy

Initial acute GVHD therapy was standard therapy with prednisone 60 mg/m^2 administered in a divided dose 3 times a day (TID). Cyclosporin was continued in patients who were already receiving it (67% of patients) or FK506 was given (2% of patients). Steroid creams were applied TID to affected areas. Steroid resistance was defined as GVHD progression after 4 days or no improvement after 7 days of steroid therapy. Patients with limited skin (grade I) GVHD were eligible for the study if they failed to improve after 7 to 14 days of prednisone therapy.

Equine ATG Therapy

Equine ATG (Atgam; Pharmacia, Peapack, NJ) therapy for steroid-resistant acute GVHD consisted of a dose of 15 mg/kg intravenously over 3 hours given twice daily for a total of 5 days or 10 doses. Patients were premedicated with acetaminophen and diphenhydramine as well as prednisone, 30 mg/m^2, given orally with each dose and then continued for at least 7 days. If responses were observed, prednisone 60 mg/m^2 was continued for 7 days and then tapered over a period of 8 weeks. If there was no response after 7 to 10 days or there was progression after 14 days, the equine ATG course was repeated.

Statistical Analysis

Response to GVHD therapy at day 28 was analyzed using Pearson's chi square test. Survival rates were analyzed using the Kaplan-Meier method from the time of initiation of equine ATG. Log rank was used for comparisons within study cohorts, and Cox regression was used to determine the independent effect of these factors. A multivariate logistical regression was used for the analysis of independent effects of study variables and response. Cumulative incidence curves were calculated to estimate chronic GVHD and infectious com-

Table 1. Maximum Initial GVHD Stage at Onset of Equine ATG Therapy

	0	1	2	3	4
Skin	19%	10%	17%	53%	1%
Liver	89%	1%	4%	4%	3%
Rectal	48%	17%	10%	22%	4%
Upper GI	72%	28%			

plications. Deaths from other causes were treated as competing risks.

The study variables included:

- Pre-HSCT donor/recipient characteristics: age, sex, sex match, diagnosis, type of donor (related, matched unrelated, or mismatched unrelated), and cytomegalovirus (CMV) serostatus of the patient and donor.
- Recipient therapy: year of transplantation, GVHD prophylaxis regimen, and conditioning regimen.
- GVHD characteristics: initial grade of acute GVHD, time to onset of acute GVHD, time to therapy, and type of organ involvement.

Selected Patient Characteristics

A total of 1016 patients underwent allogeneic transplantation during the study period of August 1990 to November 1998. Of those patients, 63% developed acute GVHD. A subset of 79 patients who developed acute GVHD but had not received prior equine ATG therapy as initial therapy were included in this analysis. Twenty percent of the patients who were females received transplants from male donors. The median age was 27 years. The diagnoses included chronic myelogenous leukemia (CML) in 35% of patients, acute lymphoblastic leukemia in 22%, acute myelogenous leukemia in 14%, metabolic disorders in 9%, and others. The donor type was matched related in 41%, mismatched related in 3%, matched unrelated in 18%, and mismatched unrelated in 39%. Seventy-one percent of the patients received cyclophosphamide and total body irradiation (TBI), 13% received TBI with other chemotherapy, and 15% received chemotherapy plus or minus ATG. GVHD prophylaxis included cyclosporin and methotrexate in 58%; methotrexate, ATG, and prednisone in 13%; T-cell depletion by elutriation in 13%; methotrexate in 5%; and FK506 in 3%. The maximum initial stages of GVHD at onset of equine ATG therapy are shown in Table 1. Eighty-nine percent of the patients had no liver involvement; in the remaining patients, liver involvement ranged from mild to moderate to severe. Approximately half the patients had evidence of lower-GI GVHD, and approximately a quarter of the patients had evidence of upper-GI GVHD.

Table 2. Factors Associated with CR/PR to Equine ATG for Acute GVHD: Multivariate Logistic Regression Analysis

	Odds Ratio (95% CI)	*P*
Diagnosis		
Nonmalignant	1.0	
Acute leukemia	2.3 (0.6-9.4)	.26
CML	5.7 (1.3-24.2)	.02
Other malignancy*	8.6 (1.2-64.2)	.04
Skin involvement		
No	1.0	
Yes	4.4 (1.2-16.5)	.03

**Myelodysplastic syndrome, 7 cases; juvenile myelomonocytic leukemia, 1 case; non-Hodgkin's lymphoma, 1 case.*

Response to Treatment

Factors associated with complete response (CR) and partial response (PR) to equine ATG for acute GVHD were analyzed using multivariate logistical regression (Table 2). Scoring nonmalignant disorders as 1.0 for odds ratios (ORs), there was a 2.3 OR for factors associated with equine ATG response in patients with acute leukemia, which was not significant; a 5.7 OR for CML, which was significant; and an 8.6 OR for other malignant disorders, which was also significant. Scoring no skin involvement as 1, there was a 4.4 OR for equine ATG response in patients with skin involvement. Twenty percent of the patients achieved a CR assessed at day 28 after initiating equine ATG, 34% had PRs, 42% had no response (NR), and 2% were not evaluable because of early death. Figure 1 shows the Kaplan-Meier plot looking at day 28 CR and PR rates and 1-year survival rates for patients with CR, PR, and NR. At 1 year posttherapy, there was no difference in the overall survival in those patients who had a CR or PR versus those patients who had NR.

Similarly, in patients who received an unrelated-donor transplant, there was a 35% 1-year survival rate versus a 29% 1-year survival rate in patients who received a related-donor transplant and who had received equine ATG as first-line therapy for steroid-resistant acute GVHD (Figure 2). Interestingly, comparison of the outcome of patients who received a T-cell–replete graft with that of patients who received a T-cell–depleted graft showed a superior overall 1-year survival rate for those patients who received a T-cell–replete graft who developed acute steroid-resistant GVHD and received equine ATG therapy compared to equine-ATG–treated patients who received a T-cell–depleted graft (Figure 3). Most importantly, the early initiation of equine ATG for steroid-resistant GVHD was a critical factor in determining long-term survival outcome. Patients who received equine ATG therapy less than 2 weeks after the diagnosis of steroid-resistant acute GVHD had survival rates superior to patients in whom initiation of equine ATG therapy for steroid-resistant acute GVHD was delayed (P = .05) (Figure 4).

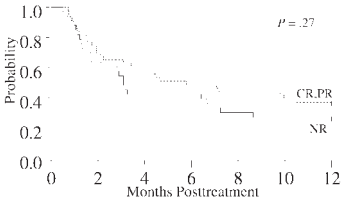

Figure 1. Kaplan-Meyer plot showing day 28 CR and PR rates and 1-year survival rates for the patients with CR, PR, and NR. At 1 year posttherapy, there were no differences in the overall survival rates in those patients with CR or PR and patients with NR.

Additional clinical risk factors associated with survival are shown in Table 3. The overall 1-year survival rate was 32%; there was 38% survival in the pediatric

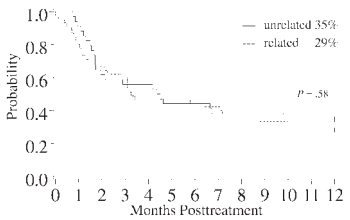

Figure 2. Similar outcomes in related- and unrelated-donor transplant recipients treated with equine ATG for steroid-resistant acute GVHD.

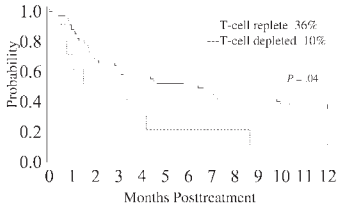

Figure 3. Survival rates in recipients of T-cell–replete donor grafts who were treated with equine ATG therapy for steroid-resistant GVHD were superior to survival rates in recipients of T-cell–depleted donor grafts.

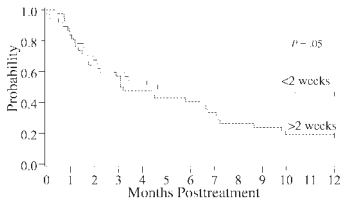

Figure 4. Early initiation of ATG for steriod-resistant acute GVHD results in superior survival.

population and 21% survival in the adult population. There was no difference in overall 1-year survival rates related to diagnosis, and there was only a suggestion of difference, which was not significant, between survival rates in patients who received TBI-containing regimens and those who did not. Comparison of survival in patients who received transplants from related donors with those who received transplants from matched unrelated or mismatched unrelated donors resulted in a *P* value of only .1. Because this study series was limited, more patients will be required to sort out whether survival rates in the patients receiving matched unrelated donor transplants or those in patients receiving mismatched donor transplants differ from those in patients receiving related donor transplants. However, the preliminary data do not suggest that these prognostic variables are important.

Table 3. Additional Clinical Risk Factors Associated with Survival

	No. of Patients	1-Year Survival	*P*
Overall	79	32%	
Age			
<18	34	38%	
≥18	45	21%	.16
Diagnosis			
Acute leukemia	28	36%	
CML	28	29%	
Nonmalignant	14	36%	
Other malignancies	9	22%	.63
Conditioning			
TBI	66	29%	
Non-TBI	13	46%	.23
Type of HSCT			
Related	34	35%	
Matched unrelated donor	14	14%	
Mismatched unrelated donor	31	35%	.10
GVHD Prophylaxis			
Methotrexate-containing	14	43%	
Cyclosporin-containing	53	34%	
T-cell depletion	10	10%	.11
Initial GVHD Grade			
1	7	29%	
2	38	42%	
3-4	34	21%	.29
Skin Involvement			
No	15	13%	
Yes	64	36%	.12
Time to GVHD Onset			
<4 weeks	44	36%	
≥4 weeks	35	26%	.14
≤2 weeks	37	46%	
>2 weeks	42	19%	.05
Response to ATG			
CR	16	38%	
PR	27	37%	
NR	33	27%	

Methotrexate-containing regimens for GVHD prophylaxis resulted in a 43% and cyclosporin-containing regimens a 34% 1-year survival rate, but most patients received both regimens. Patients who received T-cell–depleted marrow, as mentioned previously, had a 10% 1-year survival rate; only 10 patients were available for this analysis. For initial grade of GVHD, patients that had grade 1, 2, or 3-4 did not have significant differences in their overall 1-year survival rates. Additional risk factors in this analysis included no skin involvement versus skin involvement; patients with skin involvement did not have a significantly higher response rate, although the was evidence suggesting a modestly higher response rate. For patients who received second-line therapy, there was no difference in patients whose time to GVHD onset was less than 4 weeks versus those whose time to onset was greater or equal to 4 weeks. However, for patients who received second-line therapy 2 weeks or earlier after the diagnosis of acute GVHD, the overall survival rate was 46% compared to only 19% in patients receiving second-line therapy more than 2 weeks from the time of GVHD onset. Moreover, there were no differences in the 1-year survival rates depending on initial CR, PR, or NR.

Clinical risk factors associated with mortality grading are summarized in Table 4. Ranking no T-cell depletion as a 1.0 relative risk of death, T-cell depletion resulted in a 2.5 higher risk of death in the series, although a very limited number of patients were included in this series. There was no difference in relative risk related to donor type. The time to onset of GVHD had a relative risk of 1.2 per week. The time from initial therapy to equine ATG therapy per week had a 2.1 risk, a result that was significant. For skin involvement, there was no difference.

Table 4. Clinical Risk Factors Associated with Mortality: Multivariate Analysis

	Relative Risk of Death (Range)	*P*
Prophylaxis for GVHD		
No T-cell depletion	1.0	
T-cell depletion	2.5 (1.1-5.5)	.03
Type of donor		
Related	1.0	
Unrelated	0.9 (0.5-1.7)	>.8
Time to acute GVHD onset, wk	1.2 (1.0-1.4)	.05
Time from initial treatment to ATG, wk	2.1 (1.1-3.9)	.02
Skin involvement		
No	1.0	
Yes	0.7 (0.4-1.3)	.26

In summary, equine ATG resulted in a 54% overall response rate on day 28 after therapy initiation. The response rates were highest in patients with malignant disorders, especially CML, and in patients with skin involvement. The use of equine ATG as second-line therapy for steroid-resistant GVHD resulted in a 32% 1-year survival rate, which was significantly higher in patients receiving early therapy after initiation of steroids. The relative risk of death in acute GVHD was increased in patients receiving T-cell–depleted donor grafts in this limited series and in patients in whom equine ATG salvage therapy was delayed. Our conclusion is that equine ATG should be considered early in the course of therapy for steroid-resistant acute GVHD.

Acknowledgments

This study was conducted by Dr. Margaret MacMillan. Other collaborators involved in the study were Dr. Daniel J. Weisdorf, Stella Davies, Linda J. Burns, Norma K. C. Ramsay, John E. Wagner, and Todd E. DeFor.

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Tom Spitzer, MD

The Role of Equine ATG in Nonmyeloablative Stem Cell Transplantation

Nonmyeloablative stem cell transplantation is a promising therapy and an attempt to avoid some of the harsh toxicities of conventional bone marrow transplantation. Multiple potential advantages of this therapy have been observed in preclinical animal models and in the clinical setting. These advantages include reductions in transplantation-related morbidity and mortality and, most convincingly in the preclinical models, a reduction in graft-versus-host disease (GVHD). Convincing data have also been presented showing reduction in the risk of acute GVHD following nonmyeloablative conditioning therapy, and there is evidence for improved immunocompetence, at least as measured by earlier recovery of immune-effector cells posttransplantation. The data regarding rates of infectious complications in nonmyeloablative transplantation compared to conventional transplantation are currently controversial. Although there are multiple different nonmyeloablative preparative regimens, they can be divided into 2 general groups (Table 1). In the first group, mixed chimerism is reliably induced following low-dose total body radiation (TBI) alone, for example, or following our own regimen of antithymocyte globulin (ATG) and cyclophosphamide (CY) with bone marrow. These regimens are complicated by a higher risk of graft loss, less GVHD, and a higher probability of donor leukocyte infusion (DLI) use to convert the chimerism to full donor hematopoiesis. The second group of regimens, which use fludarabine (FLU) in combination with TBI, busulfan (BU), melphalan (MEL), or CY, for example, or our own newer attempt using the same conditioning regimen with peripheral blood stem cells (PBSCs), have a lower risk of graft loss than the first group but a probable higher risk of GVHD and a lower probability of using DLI for chimerism conversion. It might seem that the second group of regimens is preferable, given the higher probability of sustained engraftment. This advantage could be offset by a higher risk of GVHD, however, and there is at least the possibility that there is an enhanced antitumor effect delivered by DLI given to convert mixed chimerism to full donor hematopoiesis.

Table 1. General Groups of Nonmyeloablative Preparative Regimens

	TBI (PBSC) CY/ATG/Thymic X-ray (Bone Marrow)	FLU/TBI or BU or MEL or CY CY/ATG/Thymic X-ray (PBSC)
Graft loss	20%-30%	<10%
GVHD	20%-30%	30%-70%
DLI	30%-50%	10%

Treatment Protocol

Our clinical trial was based on an animal model in which mixed chimerism is reliably induced in mice following a nonmyeloablative preparative regimen consisting of either low-dose TBI or CY in addition to in vivo T-cell–depleting anti-CD4 and -CD8 antibodies. These mice are remarkably resistant to the induction of GVHD following DLI beginning as early as day 35 posttransplantation, and their mixed chimerism converts to full donor hematopoiesis despite this lack of GVHD. We designed a clinical protocol, based on this animal model, using CY for nonmyeloablative immunosuppression and cytoreduction, equine ATG for in vivo donor and host T-cell depletion, and cyclosporin for postgrafting GVHD prophylaxis. The protocol also included thymic radiation therapy—although it is unclear clinically what role thymic irradiation has had—and delayed DLI with conversion of mixed chimerism to full donor chimerism. We also hoped to confine the GVH response to the so-called lymphohematopoietic space.

Our equine ATG regimen consisted of CY 50 mg/kg on days –5, –4, and –3; ATG in most patients on days –1, +1, +3, and +5; thymic irradiation in patients who had not previously received mediastinal radiation therapy; cyclosporin beginning on day –1 with rapid taper to discontinuation by day 35 in patients without GVHD and with evidence of mixed chimerism; and then DLI, which was originally given as early as day 35 posttransplantation but has now been pushed out to day 49 at the earliest to allow for a 2-week washout period after discontinuation of cyclosporin.

Patients and Results

Fifty patients with HLA-matched donors have received this stem cell transplantation regimen following nonmyeloablative therapy (Table 2); 7 patients received transplants of PBSCs in the newest iteration of our protocol, and 43 received bone marrow. All of the patients had hematologic malignancy. The majority of patients had non-Hodgkin's lymphoma (NHL), and the most prevalent type of NHL was refractory B–large cell lymphoma. Overall, 39 patients (78%) had chemorefractory disease; 7 (14%) were in an untreated relapse following a prior autologous transplantation. Eighteen of the 50 patients were eligible for rapid and early discontinuation of cyclosporin and "prophylactic" DLI designed to convert their chimerism. Only 1 of the 18 patients died from a transplantation-related complication. Seven of the 18 patients did develop GVHD following DLI. Overall, 10 of the 18 patients converted to full donor chimerism, but 7 of 18 patients lost their graft. This rate of graft loss is fairly high but probably reflects the fact that a number of these patients with mixed chimerism were losing their grafts at the time of the DLI, and the DLI was unsuccessful in salvaging their chimerism. Figure 1 shows the median percentage of donor cells over time posttransplantation, with a relatively gradual but complete conversion in the majority of patients to full donor hematopoiesis and roughly parallel changes in chimerism conversion in both the $CD3^+$ and $CD3^-$ cell fractions.

One patient with a refractory follicular mixed lymphoma underwent autologous stem cell transplantation for recurrent disease in 1994. In 1998, because of chemorefractory disease, complete marrow replace-

Table 2. Patient Characteristics

No. of patients	50
Median age, y (range)	44 (22-62)
Female/male	22/28
PBSC/bone marrow	7/43
Disease	
Acute lymphoblastic leukemia	1
Acute myelogenous leukemia	6
Chronic lymphocytic leukemia	4
Hodgkin's disease	8
Multiple myeloma	3
Non-Hodgkin's lymphoma	28
Chemorefractory	39 (78%)
Untreated relapse	7 (14%)
Prior autologous transplantation	14 (28%)

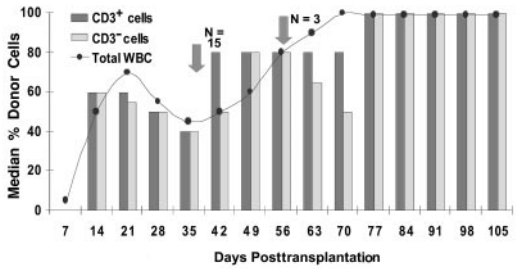

Figure 1. Mixed lymphohematopoietic chimerism after prophylactic DLI.

ment, and severe pancytopenia, he underwent a nonmyeloablative HLA-matched sibling donor transplantation. The patient has remained in continuous complete remission without evidence of acute or chronic GVHD for 4 years. Bone marrow samples before (Figure 2A) and after (Figure 2B) the 1998 treatment are shown.

Treatment results thus far raise the possibility that a more potent graft-versus-leukemia effect is captured by giving prophylactic DLI and converting chimerism. The transplantation-related mortality at 100 days was 4% overall. Grades 2 through 4 GVHD rates after the transplantation were 10% overall, and this result does not take into account the DLI. GVHD was 26% for marrow recipients and, so far, 67% for PBSC recipients. Graft loss occurred in 30% of marrow recipients and only 1 of 6 PBSC recipients thus far. Overall, antitumor response was 44%, including a 34% complete response rate. The event-free survival rate of 28% is encouraging among this group of patients with refractory predominantly intermediate-grade non-Hodgkin's lymphomas.

Induction of Tolerance for Solid Organ Transplantation

Another exciting potential application of this treatment strategy is for the induction of tolerance for solid organ transplantation. A number of preclinical observations have shown that sustained donor-specific allotolerance can be induced following the induction of mixed chimerism, even if only transiently. Using these observations as a basis, we had an opportunity approximately 3½ years ago to treat a patient with multiple myeloma and end-stage renal disease with combined HLA-matched bone marrow and kidney transplantation. The preparative regimen for this patient and subsequent patients has been very similar to the one described above. It instead involves 2 doses of cyclophosphamide at 60 mg/kg followed by hemodialysis 14 hours after each dose; equine ATG on a similar schedule; cyclosporin discontinuation somewhat later, beginning at day 60 posttransplantation; and for patients eligible because of the lack of GVHD and mixed chimerism, DLI starting 2 weeks later. The first patient in the series who received the combined bone marrow and kidney transplant had prompt normalization of serum creatinine and, despite discontinuation of cyclosporin as the sole immunosuppressant, has maintained a normal renal function for approximately 3½ years posttransplantation. As far as antitumor response is concerned, her kappa light chain as measured in the urine became undetectable except for 2 small amounts of transient kappa light chain excretion within the first year, and at 3½ years this patient had normal bone marrow and no detectable M protein. We have subsequently treated 2 more patients who are doing similarly well, and we will have an opportunity to treat more patients as part of a multicenter Immune Tolerance Network–funded clinical trial.

Conclusions

Based on the early results of nonmyeloablative stem cell transplantation for advanced malignancy and for the reduction of donor-specific allotolerance, the following conclusions can be made.

1. Lymphohematopoietic chimerism is reliably achieved following cyclophosphamide and equine ATG–based nonmyeloablative conditioning and HLA-matched donor stem cell transplantation.
2. Prophylactic DLI are associated with a high rate of chimeric conversion and antitumor response.
3. PBSC transplantation following this nonmyeloablative conditioning regimen is associated with earlier and more complete donor chimerism than is bone marrow transplantation; however, PBSC treatment has been complicated by a higher rate of GVHD.
4. Donor-specific allotolerance is achievable following a similar nonmyeloablative preparative regimen and combined bone marrow and kidney transplantation.
5. Controlled comparisons of conditioning regimens must be conducted to evaluate whether one regimen is superior to another.

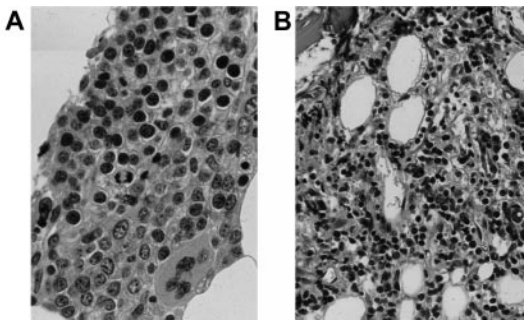

Figure 2. Bone marrow samples from a patient with stage IV B-cell non-Hodgkin's lymphoma who received a nonmyeloablative HLA-matched donor transplant for recurrent chemorefractory disease. A, Pretreatment bone marrow demonstrating hypercellularity with diffuse involvement by predominantly small-to-medium–sized cleaved lymphoma cells. B, Posttransplantation (day +106) bone marrow showing trilineage hematopoiesis with no evidence of lymphoma.

Mary Laughlin, MD

Hematopoietic Engraftment and Survival after Unrelated Donor Umbilical Cord Blood Transplantation in Adults

This report describes a retrospective analysis of the use of umbilical cord blood (UCB) single-unit grafts in patients receiving full myeloablative conditioning. This analysis included patients enrolled in phase I trials at 5 institutions during the period of February 1995 to September 1999. Patients eligible for this trial included patients with high risk or recurrent hematologic malignancies, severe aplastic anemia, or inherited metabolic or immune disorders. Criteria included age younger than 55 years and normal organ function. Another requirement was that patients have neither an available HLA-matched sibling donor nor an available unrelated donor located via the National Marrow Donor Program search. For many of the eligible patients, their disease status precluded time to identify a potential blood donor.

In this series, preferred UCB units were those that were matched at 3 of 6 major loci. Minimum cell dose was 10 million cells/kg recipient body weight. No graft manipulation was performed other than hydroxyethyl starch red cell depletion. Patients received a total-body irradiation or busulfan-based preparative regimen, and equine antithymocyte globulin (Atgam; Pharmacia, Peapack, NJ) serotherapy was administered in all conditioning. There was some variability among institutions, and total doses of ATG ranged from 60 to 90 mg given over either 2 or 3 days. The patients received graft-versus-host disease (GVHD) prophylaxis that included cyclosporin and steroids. Again, there was variability among institutions as to the dose of steroids. Steroids were generally tapered by 8 weeks after transplantation. Supportive care in all patients included administration of granulocyte colony-stimulating factor (G-CSF) beginning on day 0 and continuing until full myeloid recovery was attained. There was some variation among institutions in the G-CSF dosage, which ranged from 5 to 10 μg/kg per day.

HLA matching in this series, beginning in 1995, included serologic typing for class I and initial low resolution molecular typing for DRB1. Subsequently, confirmatory typing incorporating high-resolution analysis was used for the DRB1 locus. Scoring results of patient grafts showed that grafts were matched at 6 of 6 loci in 2 patients, 5 of 6 loci in 18 patients, 4 of 6 loci in 37 patients, and 3 of 6 loci in 11 patients. The majority of patients, 48 (71%), received UCB grafts that were disparate at 2 or more HLA antigens, and approximately half the patients received grafts that were DRB1 incompatible.

Patient Characteristics

Characteristics of the study patients are outlined in the Table. The median patient body weight in this series was 69 kg (range, 41-116 kg). The median age was 31 years (range, 18-58 years). Patient disease characteristics were typical for phase I trial enrollment; these were very high-risk patients. Fifty of the 68 patients who were scored using IBMTR (International Bone Marrow Transplant Registry) criteria were considered high or intermediate risk. Malignant disease had been diagnosed in 54 of the patients and nonmalignant disease in 14 patients. Prior autologous bone marrow transplantation had failed in 7 patients. Acute leukemias were the predominant disease entities in this series, and often the patients' disease status precluded the necessary time to identify and mobilize a matched unrelated donor. There were 15 patients with acute lymphocytic leukemia (ALL) and 19 patients with acute myelocytic leukemia (AML), including AML arising from myelodysplasia and therapy-related AML. Fifteen patients had chronic myelogenous leukemia (CML). Ten of these 15 patients had accelerated or blast crisis, and 5 were in chronic phase. All of the chronic phase patients were more than 1 year from diagnosis.

UCB Graft Characteristics

Median cryopreserved cell dose in this adult series was 2.1×10^7/kg and ranged from 1 to 6.3×10^7/kg. Infused cell dose was approximately 20% less, with a median dose of 1.6×10^7/kg and ranging as low as 0.6×10^7/kg. Median CD34 dose infused was 1.2×10^5/kg. The lower limit in this range was 0.2×10^5/kg, and, importantly, the patient who received the lowest dose grafted. CD3 was infused at a median of 4.6×10^6/kg—keep in mind these grafts were not manipulated prior to infusion—and the range was 0.9 to 9.1×10^6/kg. Chimerism in these patients was evaluated using 1 of 3 techniques: fluorescence in situ hybridization for the Y chromosome in patients receiving sex-mismatched transplants, DRB1 and male-specific hybridization in cases in which the donor and the patient differed at that locus, and quantitative polymerase chain reaction for microsatellite markers.

Engraftment Results

The definition of primary graft failure was absence of donor-derived myeloid engraftment as defined by an absolute neutrophil count (ANC) greater than 500 on the first of 3 consecutive days, by day +42 in patients surviving more than 28 days after transplantation.

The UCB cryopreserved cell dose of the graft affected speed of engraftment, with higher cell dose predicting faster engraftment, as indicated in Figure 1. The median day to neutrophil recovery was 24 days in the patients receiving the higher cell dose compared with 32 days in the patients receiving a lower cell dose. All the patients who demonstrated engraftment showed complete donor chimerism, and there were no late graft failures. The median day to neutrophil recovery for all patients in this series was 27 days (range, 13-59 days). In 3 patients, engraftment occurred after day 42, and therefore these patients did not meet criteria for engraftment. Two of these

Adult UCB Recipients: Patient Characteristics

Median weight 69 kg (range, 41-116 kg)
Median age 31 y (range, 18-58 y)
Disease characteristics
- 50/68 Patients high risk according to IBMTR criteria
- 54 Cases malignant disease and 14 nonmalignant
  - (7 failed prior BMT)
    - 15 Acute lymphocytic leukemia
    - 19 Acute myelogenous leukemia
    - 15 Chronic myelogenous leukemia
    - 1 Chronic myelomonocytic leukemia
    - 1 Blackfan-Diamond anemia
    - 1 Adrenoleukodystrophy
    - 4 Fanconi anemia/myelodysplastic syndrome
    - 2 Refractory/recurrent Hodgkin's disease
    - 1 Chronic lymphocytic leukemia
    - 1 Non-Hodgkin's lymphoma
    - 2 Myelodysplasia
    - 4 Severe aplastic anemia
    - 2 Myelofibrosis

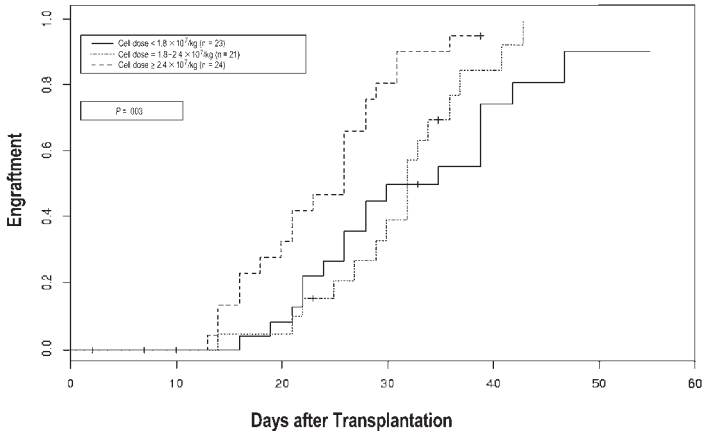

Figure 1. Donor engraftment versus UCB cell dose.

patients subsequently died and 1 patient was alive as of this report, 3 years posttransplantation. The probability of neutrophil recovery was 90% in this series, with a confidence interval (CI) of 85% to 100%. Median day to platelet recovery was delayed, 58 days, and ranged out to 142 days. Median day to platelet recovery greater than 100,000 was 124 days. Median time to hemoglobin transfusion independence was approximately 2 months.

Analysis of donor engraftment versus HLA match of the graft indicated that there were no significant differences in rates or kinetics of engraftment based on HLA disparity. Because we were concerned that perhaps clinicians might choose a higher cell dose graft over a better match graft, we did an analysis of days to ANC 500 versus prethaw cell dose for patients who received 4-to-6–matched grafts, patients who received lesser matched grafts, and patients who received better matched grafts. These results indicated that there was no significant trend. The 4-to-6–matched grafts spanned broad cell dose in this analysis.

Trial Outcomes

There were 8 patients who experienced early death. Graft failure was observed in 5 of the 60 surviving patients. The incidence of severe grade 3 acute GVHD was 20% (CI, 10%-30%). The incidence of chronic GVHD in these adults was 38% (CI, 20%-50%). The chronic GVHD we have observed differs dramatically from that in our patients receiving grafts from matched unrelated donors. For example, we did not observe sclerodermal type skin involvement. The patients generally had sicca symptomatology and/or sinusitis. In all but 1 patient, the chronic GVHD was limited and nonprogressive. Because all patients received ATG pretransplantation as part of conditioning, the effect of ATG on rates of donor-derived hematopoietic engraftment and incidence of GVHD could not be determined.

The median follow-up of survivors in this series was 24 months and ranged out to 53 months. There were only 4 relapses in this series of very-high-risk patients, and all relapse events occurred in the first year, at 3, 6, 10, and 11 months posttransplantation. Relapsing patients included 3 patients with ALL who were Philadelphia-chromosome positive and 1 patient with Hodgkin's disease whose disease progressed posttransplantation. Event-free survival (EFS) at 48 months was 26% in this series (CI, 18%-35%); this result represents 18 patients.

Analysis of survival rates in relation to HLA match or mismatch of the graft is shown in Figure 2. The patients who received grafts matched at 3 of 6 loci did not fare well, but the patients who received a graft matched at 4 of 6 loci did better than patients receiving better matched, 5 of 6 or 6 of 6, grafts. This observation for the adult patients is not reflective of the larger series of pediatric patients. Although these results did not attain statistical significance, they indicate that in adult recipients who received a dramatically lower CD34 dose, immunologic factors in the graft accessory populations may mediate survival rates, a possibility that we are actively investigating.

Comparison of patient survival in relation to diagnosis indicated that there were no significant differences; however, there were trends (Figure 3). Patients with CML had EFS rates in the 40% range, whereas patients with ALL had EFS of 20%. We did additional studies of EFS versus the graft CD34 infused dose (Figure 4). For clinicians caring for patients undergoing UCB transplantation, CD34 information on the graft has thus far not been routinely available, and the clinician has had to rely on the nucleated cell dose and HLA matching in choosing a graft for a particular patient. We observed a CD34 content

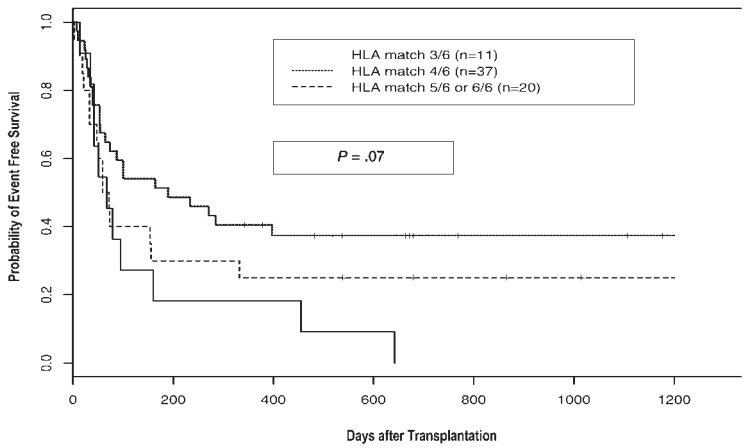

Figure 2. Survival curves by HLA match.

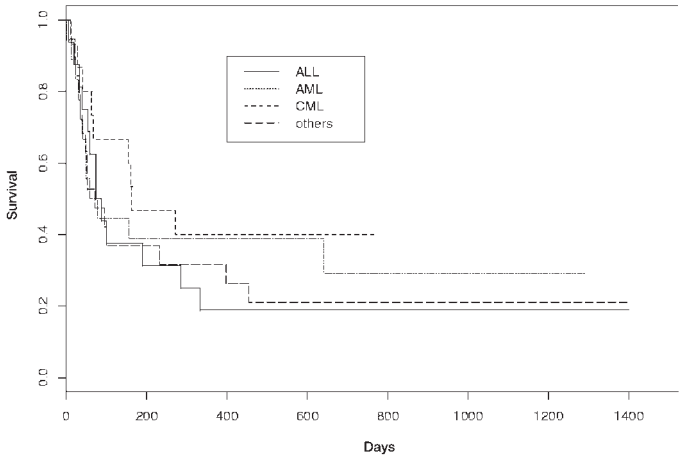

Figure 3. Survival curves by diagnosis.

of the graft measured on day 0 at the time of thaw correlated with cryopreserved cell dose, and, in this series of adult patients, those patients receiving a higher CD34 infused cell dose, greater than 1.2×10^5/kg, demonstrated improved EFS rates compared to patients receiving a lower CD34 cell dose.

Our group has done additional studies, as have others, to assess the kinetics of immune recovery in these patients. The absolute lymphocyte count was noted to normalize by 1 year posttransplantation. Additional studies to assess T-cell populations have noted a paucity of T-cells during the early posttransplantation period. Even at 1 year after transplantation, these T-cell populations are one third those of normal controls. The early posttransplantation period is marked by predominance of natural killer cells. Another interesting phenomenon is the B-cell rebound phenomenon, which has also been described by our European colleagues, beginning at 6 months posttransplantation and continuing to 1 year posttransplantation. During this rebound, B-cell populations can comprise 6-fold higher levels than those of healthy adult controls.

At our institution, we have done further studies in these trial patients in which we have analyzed emerging donor lymphocytes at day 100 after transplantation, testing them in a mixed lymphocyte culture (MLC) against recipient cells collected pretransplantation versus a third-party HLA-disparate cell line. We have also tested reactivity against cytomegalovirus. What we have observed in these patients is recipient-specific tolerance exerted by the emerging donor lymphocytes. Importantly, levels of interferon-γ and other type I cytokines produced by these lymphocytes in MLC are lower in the presence of the recipient cells and higher in the presence of third-party irrelevant targets, indicating a recipient-specific tolerance rather than a pan-immunosuppression. Results of further studies including thymidine incorporation have correlated with the low proliferative rates of these emerging donor cells in the presence of the recipient cells and high proliferative rates in the presence of third-party irrelevant targets; in addition, T-helper 2–cytokine production, interleukin (IL)-4 and IL-10, is high in the presence of recipient targets and low in the presence of third-party irrelevant targets.

Conclusions

In summary, we feel that UCB can successfully engraft in adults with high-risk or refractory hematologic malignancies and marrow failure syndromes despite a high level of HLA disparity. UCB from unrelated donors is associated with a low incidence of severe and acute chronic GVHD. We and others have observed delayed time to hematopoietic and immune recovery. These UCB transplantation procedures in adult recipients, using single units and full ablation, have been marked by a high day-100 mortality, in the range of 50%. Of those 50% of patients, approximately half died of regimen-related toxicity and half died of infectious complications.

Acknowledgments

Collaborators in these studies were Juliet Barker and John Wagner from the University of Minnesota; Barbara Bambach from Roswell Park Cancer Institute; Omer Koc, Hillard Lazarus, and Stan Gerson from Case Western; David Rizzieri and Joanne Kurtzberg from Duke University; Mitch Cairo from Columbia Presbyterian; and Cladd Stevens and Pablo Rubinstein from the New York Blood Center.

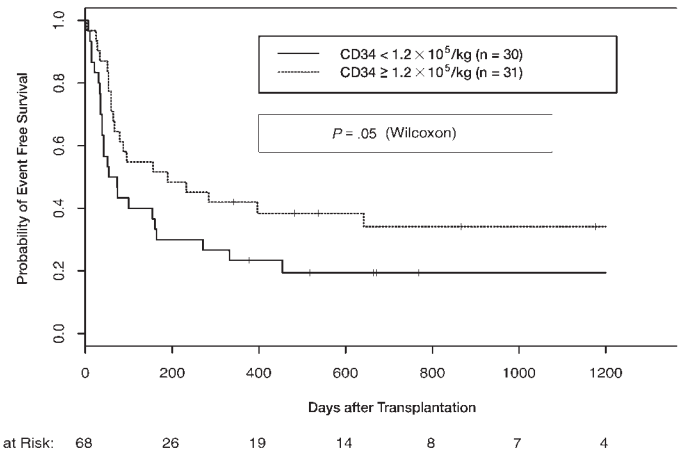

Figure 4. Survival curves by graft CD34 dose.

JOURNAL Watch

A scan of recent medical literature identified these articles of special importance in the science and clinical application of blood and marrow transplantation.

Liu J: Selective T-cell subset ablation demonstrates a role for T1 and T2 cells in ongoing acute graft-versus-host disease: a model system for the reversal of disease. Blood 98:3367-3375, 2001.

A new model was developed for simultaneous investigation of the mechanisms of graft-versus-host disease (GVHD) after allogeneic stem cell transplantation and the efficacy of herpes simplex virus thymidine kinase (HSV-tk) suicide gene-based T-cell depletion. Donor T cells were derived from splenocytes of mice in which the HSV-tk transgene was controlled by the interleukin (IL)-2 (IL-2-tk) or IL-4 (IL-4-tk) promoter. This provided a means of studying the contributions of both T1 and T2 cells to ongoing GVHD reactions in a minor histocompatibility antigen-mismatched setting. Recipient animals started ganciclovir treatment at 14 days after transplantation, allowing evaluation of the effects of treatment for established GVHD.

In both IL-2-tk and IL-4-tk recipients, ganciclovir treatment was followed by significant clinical improvement, as evidenced by weight gain and improved survival. Histologic disease improved partially, and the frequency of specifically targeted cytokine-secreting cells decreased. Memory T-cell accumulation decreased as well, suggesting that GCV was killing activated, dividing, and alloreactive T cells.

The findings support the involvement of both T1 and T2 cells in the ongoing process of acute GVHD after allogeneic stem cell transplantation. In the study model, it is possible to reverse even advanced GVHD by ganciclovir ablation of tk-expressing T cells. The study provides the first controlled data supporting the use of selective ablation of T-cell subsets for clinical treatment of GVHD.

Fox-Geiman MP, Fisher SG, Kiley K, et al: Double-blind comparative trial of oral ondansetron versus oral granisetron versus IV ondansetron in the prevention of nausea and vomiting associated with highly emetogenic preparative regimens prior to stem cell transplantation. Biol Blood Marrow Transpl 7:596-603, 2001.

Oral granisetron and oral ondansetron are equally effective as IV ondansetron in preventing cisplatin-induced emesis. However for patients undergoing BMT, the optimal route and schedule of serotonin antagonist therapy remain to be determined. This randomized trial compared three strategies for prevention of nausea and vomiting in patients receiving preparative therapy for stem cell transplantation.

The double-blind study included 102 patients receiving preparative therapy with high-dose chemotherapy or chemoradiotherapy before stem cell transplantation. Patients were randomized to receive oral ondansetron, 8 mg every 8 hours; oral granisetron, 1 mg every 12 hours; or IV ondansetron, 32 mg every 24 hours. Each regimen was given each day during preparative treatment and 1 day afterward; all three groups also received IV dexamethasone, 10 mg/d.

Rates of complete response—defined as no emesis with absent or mild nausea, with or without rescue antiemetics—were 48% with oral ondansetron, 47% with oral granisetron, and 49% with IV granisetron. The three groups also had similar rates of major efficacy, defined as no more than one episode of emesis or major nausea, with or without rescue antiemetics: oral ondansetron 82%, oral granisetron 84%, and IV ondansetron 81%.

The three groups also had similar mean visual analog scale nausea scores, with a range of 27 to 32 mm on a 100-mm scale. Including rescue antiemetics, total costs of antiemetic therapy were $641 with oral ondansetron, $770 with oral granisetron, and $1,747 with IV ondansetron.

Oral granisetron, oral ondansetron, and IV ondansetron are similarly effective in the prevention of nausea and vomiting among patients undergoing preparative therapy before stem cell transplantation. Of the three regimens, oral ondansetron is the most cost-effective.

Montagna D, Maccario R, Locatelli F, et al: Ex vivo priming for long-term maintenance of antileukemia human cytotoxic T cells suggests a general procedure for adoptive immunotherapy. Blood 98:3359-3366, 2001.

This study evaluated approaches to ex vivo priming of purified CD8 lymphocytes for adoptive immunotherapy in patients with acute myeloid leukemia (AML). The study included 4 pediatric AML patients and their allogeneic BMT donors. Different parameters were manipulated to determine the best approach to in vitro priming of naïve CD8 cells.

Dendritic cells induced a cytotoxic T-lymphocyte (CTL) response when challenged with CD40 ligand. This response could be duplicated with interleukin (IL)-12 and increased further with IL-7. However, to create long-lived antileukemic CTLs, it was necessary to use irradiated CD4 T lymphocytes in the induction phase. With this approach, the investigators were able to generate long-term CTLs from the bone marrow of all 4 donors.

A promising ex vivo approach for induction of antitumor T-cell lines for the treatment of AML is reported. A major advantage of the new technique is that no specific tumor antigen needs to be identified; instead, the optimal combination of antigen and available T-cell repertoire can be selected. This immunotherapy approach may be of therapeutic value for a wide range of tumors and chronic infectious diseases.

JOURNAL Watch

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Taylor PA, Noelle RJ, Blazar BR: $CD4^+CD25^+$ immune regulatory cells are required for induction of tolerance to alloantigen via costimulatory blockade. J Exp Med 193:1311-1317, 2001.

The effects of $CD4^+CD25^+$ cells on T cell responses to alloantigens and on tolerance induction were studied in vitro and in vivo. The investigators incubated mouse $CD4^+$ or $CD4^+CD25^+$ T cells with alloantigen in the presence of anti-CD40 ligand (CD-40L) or anti-B7 monoclonal antibodies. This produced secondary mixed leukocyte reaction hyporesponsiveness and tolerance to alloantigen in recipient animals.

The presence of $CD4^+CD25^+$ cells appeared essential for the induction of tolerance by costimulatory blockade. When this cell population was depleted from $CD4^+$ responders, ex vivo tolerance induction to alloantigen was completely eliminated—responses to alloantigen restimulation continued both in vitro and in vivo. When $CD4^+CD25^+$ cells were re-added to $CD4^+CD25^-$ cultures, tolerance induction returned.

In the study model, the $CD4^+CD25^+$ cell population appears critical to ex vivo tolerance induction via costimulatory blockade of two different pathways. The data suggest that these cells play an essential role in regulating responses to alloantigen. Efforts to induce tolerance via T cell costimulatory pathways must address the role of $CD4^+CD25^+$ cells.

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