

# BLOOD AND MARROW TRANSPLANTATION

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## REVIEWS

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### IN THIS ISSUE

- INTRODUCTION** 1  
*John R. Wingard, MD*
- MEMBERSHIP APPLICATION** 2
- ASBMT NEWS** 3
- SYMPOSIUM REPORT** 4  
**New Strategies for the Prevention of *Pneumocystis carinii* Pneumonia and Other Opportunistic Infections after Stem Cell Transplantation**  
**2002 Tandem BMT Meeting**  
**February 2002, Orlando, Florida**  
*Thomas R. Spitzer, MD*  
*Paolo Anderlini, MD*
- NONMYELOABLATIVE REGIMEN ALLOWS PERIPHERAL EXPANSION OF DONOR T-CELLS** 11  
*Nelson J. Chao, Cong X. Liu, Barbara Rooney, Benny J. Chen, Gwynn D. Long, James J. Vredenburgh, Ashley Morris, Cristina Gasparetto, David A. Rizzieri*
- GVHD TREATMENT WITH STEROIDS** 13  
*Margaret L. MacMillan, Daniel J. Weisdorf, John E. Wagner, Todd E. DeFor, Linda J. Burns, Norma K.C. Ramsay, Stella M. Davies, Bruce R. Blazar*
- JOURNAL WATCH** 15

### Introduction

#### When Is an Ounce of Prevention Better?

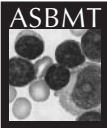
by John R. Wingard, MD, Editor

Tremendous strides have been made in minimizing the adverse effects of infection after hematopoietic stem cell transplantation (HSCT) over the past several decades. Sequentially, risk factors for various infectious complications have been identified; the nature of deficits in host defenses and their change over time have been characterized; new antimicrobial agents have supplanted older, more toxic, or less efficacious ones; and clinical trials to define the most effective ways to quell morbidity have been conducted. Prevention is intuitively appealing, but in the realm of microbial diseases is fraught with potential danger. The chief hazard is the emergence of drug resistance, which has plagued antimicrobial therapies for decades. Accordingly, infection prophylaxis should be used judiciously: only in settings where the strategy has been found to be effective and offers advantages over other treatment approaches. Moreover, prophylaxis should be monitored over time, because a strategy that is effective one day may become useless the next with the emergence of drug resistance.

Several years ago the Centers for Disease Control and Prevention convened a group of infectious disease and HSCT experts to discuss the threat of infectious morbidity after HSCT and to review what available evidence there was to support various infection prophylaxis strategies. This combined effort represented a unique and important opportunity to codify the state of knowledge and standardize practices. The result was a document endorsed by the American Society for Blood and Marrow Transplantation, the Infectious Disease Society of America, and the Centers for Disease Control and Prevention. It was published as a supplement to *Biology of Blood and Marrow Transplantation* (2000;7(6a):1-95) and a shorter version was published in *Morbidity and Mortality Weekly Report* (2000;49(RR-10):1-125). The recommendations are available at the ASBMT Web site (<http://www.asbmt.org/policystat/policy.html>).

In the proceedings of a symposium presented at the 2002 Tandem Transplant meetings supported through an educational grant by GlaxoSmithKline, Drs. Spitzer and Anderlini briefly review these prevention guidelines. They also present some preliminary data on atovaquone, an agent that has excellent activity against *Pneumocystis carinii* and offers some toxicity advantages over trimethoprim-sulfamethoxazole. The interchange with the audience in the question-and-answer session is one of the strengths of this symposium proceeding and highlights an important lesson. Just as there are shifts in microbial pathogens and drug sensitivities, prevention guidelines also cannot remain static. There were numerous knowledge gaps in 2000 when the ASBMT/IDSA/CDC guidelines were published and there remain many today. Continued study is necessitated by changing transplantation practices, emerging pathogens, alterations in drug susceptibilities, and new diagnostic testing. New drugs (atovaquone, among others) must be fit into our practice. Novel strategies must be evaluated in clinical trials for advances to continue.





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## ASBMT News

### **Dec. 13 Housing Deadline for Tandem BMT Meetings**

The housing deadline is Dec. 13, for the 2003 Tandem BMT Meetings in Keystone, Colo. After that date, sleeping accommodations at special conference rates are on a "space available" basis.

The Tandem BMT Meetings, Jan. 30-Feb. 3, are the combined annual meetings of ASBMT and the International Bone Marrow Transplant Registry/Autologous Blood and Marrow Transplant Registry (IBMTR/ABMTR).

A Housing Reservation Form can be downloaded and printed from the ASBMT Web site at [www.asbmt.org](http://www.asbmt.org), or call the Keystone Resort reservations desk toll-free at (800) 222-0188 and mention the Tandem BMT Meetings to obtain special conference rates.

Meeting registration also is available online at the ASBMT or IBMTR/ABMTR Web sites.

### **Awards and Grants to New Investigators Exceed \$600,000**

Grants and awards totaling \$610,000 were presented to young investigators during the ASBMT President's Dinner at the recent Tandem BMT Meetings in Orlando.

Among the recipients were:

- **Guenahel Danet, PhD**, University of Pennsylvania, Philadelphia – \$25,000 renewable ASBMT/Merck New Investigator Award.
- **Yong-Guang Yang, MD**, Massachusetts General Hospital, Boston – \$25,000 renewable ASBMT/Orphan Medical New Investigator Award.
- **Subramaniam Malarkannan, PhD**, Medical College of Wisconsin, Milwaukee – \$25,000 renewable ASBMT/Roche New investigator Award.
- **Thomas Davis, MD**, Stanford University, and **Lynn Graf, PhD**, Fred Hutchin-

son Cancer Research Center – \$5,000 each for *Biology of Blood and Marrow Transplantation* Editorial Awards.

- **Erhan Gokmen, MD**, University of Texas Health Science Center, San Antonio, and **Anna Mari Malkki, PhD**, Fred Hutchinson Cancer Research Center, Seattle – \$240,000 each for the Amy Strelzer Manasevit Scholarship from The Marrow Foundation.
- **Jakub Tolar, MD, PhD**, University of Minnesota, Minneapolis – \$35,000 Boehringer Ingelheim Post-Doctoral Fellowship from The Marrow Foundation.
- **Onder Alpdogan, MD; Benny Chen, MD; Shawn Clouthier, MS; Kenneth Cooke, MD; Francis Flomerfeld, PhD; Stephanie Lee, MD; Lianne Marks, MD, PhD; Pavan Reddy, MD; Rui Sun, MD; and Catherine Wu, MD** – \$1,000 each, travel grants supported by Orphan Medical, Inc.

### **New Investigator Evaluates Techniques for Fighting Lethal Infections after BMT**

A recipient of the ASBMT/Roche New Investigator Award recently submitted a progress report on her work in enhancing the immune system's response to infections after bone marrow transplantation.

**Janice (Wes) Brown, MD**, and her team at Stanford University School of Medicine have been developing and transplanting a subpopulation of stem cells, common lymphocyte progenitors (CLPs), in mice undergoing bone marrow transplants. In her experiments, mice receiving CLPs had a dramatically lower risk of death from the cytomegalovirus virus that frequently becomes reactivated in transplant recipients. Treatment with CLPs did not lead to the development of graft-versus-host-disease.

The report prepared for the ASBMT Executive Committee indicated some

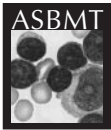
promising new approaches for combating other infections. Her studies have found that transplanting spleen cells from mice previously immunized with *Aspergillus fumigatus* fungus helped reduce the risk of death from *Aspergillus* infection after bone marrow transplantation. Mice receiving cells from immunized animals were five times more likely to survive an *Aspergillus* attack than non-immunized mice.

Transplantation with common myeloid progenitors and granulocyte-monocyte progenitors reduced the risk of death not only from *Aspergillus* but also from *Pseudomonas aeruginosa*. The administration of a new form of the antifungal drug amphotericin before bone marrow transplantation also increased the chances of surviving an *Aspergillus* attack.

The experimental techniques have used specially selected cell populations to curtail the most serious infections that may occur when the immune system is reconstituting itself after BMT. "These approaches addressed the most lethal of the common kinds of infection: viruses, bacteria, and fungi," Dr. Brown said. "Using these models of infection helps us better understand the role of specific subpopulations of immune cells in defending against these pathogens."

A goal of Dr. Brown's research is to combine several approaches for more comprehensive prevention of infections after BMT. "We're studying the effects of combinations of progenitor cells in our engineered grafts, as well as the benefit of combining graft engineering with antimicrobial medications on the outcome of these infections," Dr. Brown said.

Dr. Brown has been the recipient of \$50,000 in new investigator awards from ASBMT, supported by an educational grant from **Roche Laboratories, Inc.** The financial support is one of a series of such awards given annually by the society.



# SYMPOSIUM Report

## New Strategies for the Prevention of *Pneumocystis carinii* Pneumonia and Other Opportunistic Infections after Stem Cell Transplantation

2002 Tandem BMT Meeting

February 2002, Orlando, Florida

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Management of infections in hematopoietic stem cell transplant (HSCT) recipients is crucial, because infections are the leading cause of morbidity and mortality after transplantation [1]. Candidates who receive HSCT include those with neoplastic diseases, hematologic disorders, immunodeficiency syndromes, congenital enzyme deficiencies, and autoimmune disorders [2-5]. Antimicrobial prophylaxis is routinely practiced in HSCT patients because immunosuppressive regimens used in the process make these patients candidates for opportunistic infections (OIs).

Important advances have been made in the prevention of OIs in HSCT patients. For example, even though several studies have shown that cytomegalovirus (CMV) infections remain an independent prognostic factor in survival outcomes after allogeneic transplantation, CMV has lost its reputation as the most feared pathogen. A new and improved arsenal of drugs is also now available for treating and preventing certain fungal infections. New treatment options are available for prophylactic treatment of *Pneumocystis carinii* pneumonia (PCP) as well. However, advances in prophylaxis and treatment of OIs have been offset by other factors: treatment of older and sicker patients, use of alternative donors, the occurrence of more graft-versus-host disease (GVHD), and availability of more

immunosuppressive agents for the treatment of GVHD.

To address prevention and prophylaxis, the US Centers for Disease Control and Prevention (CDC), along with the Infectious Disease Society of America (IDSA), and the American Society for Blood and Marrow Transplantation (ASBMT) have collaborated in the development of evidence-based guidelines for preventing OIs among stem cell recipients [6].

### Phases of Immune Recovery and Infectious Complications

OIs among autologous and allogeneic HSCT patients are dependent on the time of immune recovery, which occurs in 3 phases: the preengraftment phase, the postengraftment phase, and the late phase. The preengraftment phase occurs from day 0 to day 30 after HSCT; the postengraftment phase occurs from day 30 to day 100 after HSCT; and the late phase occurs after day 100. Each phase is characterized by a susceptibility to infection correlating with the state of immune recovery that occurs during that post-HSCT time frame (Figure 1).

The timing of infections after HSCT depends on several factors, including granulocyte and lymphocyte recovery, GVHD, and the immunosuppressive regimen used. In autologous transplantations the period of neutropenia is shorter when peripheral

stem cells are used instead of bone marrow. The median time to neutrophil recovery is 9 to 12 days, greatly reducing the incidence of bacterial and other infections. Because fevers often resolve with the resolution of neutropenia, there is rarely a need to empirically treat these patients with amphotericin B. However, other factors may be important in the pathogenesis of infections in autologous HSCT recipients. Chemotherapy and radiation therapy used in the conditioning regimens often result in a breakdown of the mucosal barrier, creating a portal of entry for infectious agents. After autologous transplantation, transient T-cell dysfunction may last for 6 months or longer. Although the severity of T-cell dysfunction, at least as measured in vitro, is comparable to that seen in allogeneic transplantation, the spectrum of infections is considerably less prominent in this group of patients.

Allogeneic stem cell transplants are also associated with a neutropenic period. However, it is usually longer than that of autologous transplantation, in part due to drugs such as methotrexate that are used for GVHD prophylaxis. As with autologous transplants, altered mucosal barriers also may cause infections. Patients, especially those with GVHD, also may develop profound hypogammaglobulinemia and may need intravenous immune globulin replacement therapy. Because it is associ-

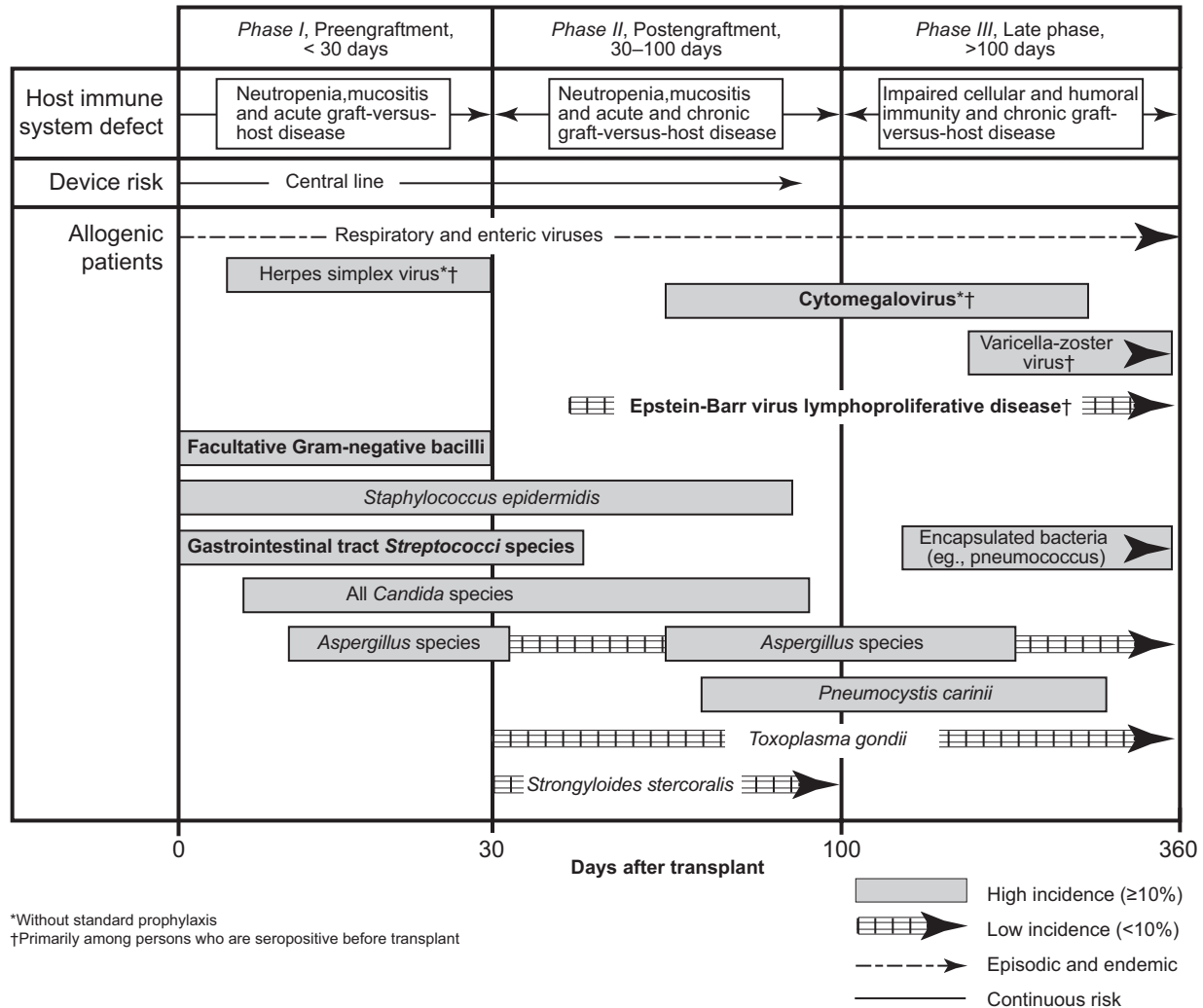


Figure 1. Phases of opportunistic infections (OIs) among allogeneic hematopoietic stem cell transplant (HSCT) recipients.

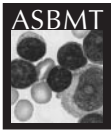
ated with a delayed immunologic recovery and prolonged immunodeficiency, GVHD is a substantial risk factor for infection among allogeneic HSCT recipients [7].

Infectious complications of HSCT are similar during the neutropenic (preengraftment) phase for patients receiving either autologous or allogeneic transplants. The risk of Gram-positive infections is higher than that of Gram-negative infections. In large measure, this shift may be attributed to the routine use of indwelling, tunneled catheters, which are potential

sources of Gram-positive organisms that colonize the skin. The increasing use of fluoroquinolones for antimicrobial prophylaxis also has contributed to a decline in Gram-negative infections. With prolonged neutropenia, fungal infections also are prevalent. Patients are at risk for reactivation of herpes simplex virus. PCP also has been observed in this group.

In allogeneic HSCT recipients, the postengraftment and late phases may be especially high-risk periods for infections, particularly in those patients with GVHD

who require prolonged use of high-dose steroids and other immunosuppressive agents. This subgroup is at a high risk for *Aspergillus* and other fungal infections. A host of viral infections is common, including CMV, herpes simplex virus, respiratory syncytial virus, and adenovirus. The incidence and severity of these infections closely correlate with the degree of immunosuppression. For example, patients may develop intractable adenovirus hemorrhagic cystitis, which may resolve on tapering and discontinuation of



**Table 1. Evidence-based Rating System Used to Determine Strength of Recommendations\***

Category	Definition	Recommendation
A	Strong evidence for efficacy and substantial clinical benefit	Strongly recommended
B	Strong or moderate evidence for efficacy, but only limited clinical benefit	Generally recommended
C	Insufficient evidence for efficacy; or efficacy does not outweigh possible adverse consequences (eg, drug toxicity or interactions) or cost of chemoprophylaxis or alternative approaches	Optional
D	Moderate evidence against efficacy or for adverse outcome	Generally not recommended
E	Strong evidence against efficacy or of adverse outcome	Never recommended

\*Adapted from CDC. 1999 USPHS/IDSA guidelines for the prevention of opportunistic infections in persons infected with human immunodeficiency virus. *MMWR*. 1999;48(RR-10):1-66.

corticosteroid or other immunosuppressive therapy.

### Infection Prophylaxis

Guidelines for treating OIs in persons infected with the human immunodeficiency virus (HIV) were first established in 1995 and later revised [8-10]. In 2000, because of their overwhelming success, the guidelines were expanded to include prevention in immunosuppressed individuals receiving HSCT [6]. Although data from prospective trials to determine the optimal prophylactic strategy in many OIs are lacking, the recommendations represent a remarkable and comprehensive evidence-based document on the prevention of OIs in HSCT patients.

The principles of the rating system were developed by the IDSA and the US Public Health Service for use in the guidelines for preventing OIs among HIV-infected persons [8-11]. Prevention strategies are rated first by the strength of the recommendation (Table 1). Strong recommendations are placed in category A and imply that there are strong evidence-based data for efficacy and clinical benefit. Category E contains drugs that are never recommended because of strong evidence against efficacy or of adverse events. A roman numeral designates the quality of the evidence supporting the recommendation, with I denoting strong evidence and III denoting lack of evidence (Table 2).

A number of studies have indicated that quinolone antibiotics decrease Gram-negative infections not only in patients with leukemia but also in patients receiving HSCT. However, this decrease has not

been correlated to a survival benefit, and the CDC guidelines therefore do not recommend the use of fluoroquinolone prophylaxis. Nonetheless, surveys have identified that fluoroquinolones are routinely used in the HSCT setting likely to prevent the potentially catastrophic Gram-negative infections that may occur during the neutropenic phase. Before using prophylactic antibiotics among asymptomatic, afebrile, neutropenic recipients, physicians should review hospital and HSCT center antibiotic-susceptibility profiles, especially when a single antibiotic is used. Fluoroquinolone resistance is a growing issue [12,13]. Vancomycin should not be used for routine bacterial prophylaxis (DIII) because vancomycin-intermediate *Staphylococcus aureus* and vancomycin-resistant *Enterococcus* (VRE) are increasing concerns [14].

To prevent invasive candidal disease, the CDC guidelines recommend the use of fluconazole, 400 mg/day, orally or intravenously for fungal prophylaxis with fluconazole-susceptible *Candida* spp (AII). This use has been substantiated in 2 separate randomized trials with bone marrow transplant recipients, which

showed a decrease in invasive fungal infections. A survival benefit was reported in one study [15,16]. Although the dosage of 400 mg/day has not been compared with lower dosages, the increasing emergence of resistance to non-albicans candidal species suggests that the higher dosage (400 mg/day) be used.

*Aspergillus* prophylaxis is also reasonable in certain high-risk groups such as patients with severe GVHD who are in need of prolonged immunosuppressive therapy. Although amphotericin B has been used in many studies to prevent aspergillosis, the data are limited, and the CDC guidelines do not recommend its use [6].

The CDC guidelines recommend acyclovir for herpes simplex virus (HSV) prophylaxis [6]. HSV infections, which are a potential cause of severe morbidity and mortality in HSCT patients, are effectively prevented with acyclovir. To prevent reactivation during the early post-transplantation period, acyclovir is recommended in HSV-seropositive allogeneic recipients (AI) and is administered with conditioning therapy and continued until engraftment or resolution of mucositis, or until approximately 30 days after HSCT (BIII). The guidelines do not recommend prophylaxis for varicella-zoster virus infections. Data from randomized controlled trials for prolonged acyclovir prophylaxis are lacking. However, the experiences from a number of transplantation centers suggest that fewer varicella-zoster virus infections occur when acyclovir prophylaxis or similar drugs are used beyond the neutropenic period. Recommendations to prevent community respiratory

**Table 2. Evidence-based Rating System Used to Determine Quality of Evidence Supporting Recommendation\***

Category	Definition
I	Evidence from at least one well-executed randomized, controlled trial
II	Evidence from at least one well-designed clinical trial without randomization; cohort or case-controlled analytic studies (preferably from more than one center); multiple time-series studies; or dramatic results from uncontrolled experiments
III	Evidence from opinions of respected authorities based on clinical experience, descriptive studies, or reports of expert committees

\*Adapted from CDC. 1999 USPHS/IDSA guidelines for the prevention of opportunistic infections in persons infected with human immunodeficiency virus. *MMWR*. 1999;48(RR-10):1-66.

viruses include exposure prevention and vaccination or chemoprophylaxis. Ganciclovir is recommended for prophylaxis or preemptive therapy of CMV infections from the time of engraftment until 100 days after HSCT (ie, phase II) (AI). Infusion of donor-derived, Epstein-Barr virus (EBV)-specific cytotoxic T-lymphocytes after T-cell depleted bone marrow transplantation is a promising new strategy in the prophylaxis of EBV-related lymphoma. The CDC guidelines do not address prophylaxis against HHV-6 and other herpes viruses, which are being recognized as potential infectious agents in HSCT recipients.

Examples of advances in pharmacologic infection prophylaxis include voriconazole, valganciclovir, and atovaquone for fungal, viral, and PCP infections, respectively. These drugs are available as oral formulations and have good bioavailability and activity against important, common pathogens in patients receiving HSCT. In a randomized trial, voriconazole was shown to be as effective as amphotericin B in the empiric treatment of persistent febrile neutropenia. It is effective for *Aspergillus* infections and is likely to replace less effective and more toxic prophylactic strategies. Valganciclovir, a reasonably well-absorbed pro-drug of ganciclovir, is an oral formulation in use for prophylaxis and preemptive therapy of CMV infections. Atovaquone (Mepron; GlaxoSmithKline, Research Triangle Park, NC) is an oral agent with no myelosuppressive properties that has been introduced for PCP prophylaxis. Mepron suspension is an oral formulation of microfine particles of atovaquone that facilitates absorption of the drug [17].

### ***Pneumocystis carinii* Pneumonia**

The incidence of PCP is estimated at 13% in HSCT recipients who have not received prophylactic treatment [18]. The optimal duration of prophylactic therapy is unknown but is approximately 6 months for most autologous recipients and 6 to 12 months for allogeneic recipients. In immunosuppressed recipients,

**Table 3. Incidence of PCP following Bone Marrow Transplantation**

Brigham and Women's Hospital Study [19]	University of Minnesota Study [20]
Patients: 451 Evaluated patients: 327 Autologous: 133 Allogeneic: 190 T-cell-depleted transplants: 58 Non-T-cell-depleted transplants: 132 Incidence of PCP: 2.4%	Patients: 1454 PCP cases: 19 (10 were late posttransplantation) Incidence of PCP: 1.3% PCP survival: 37%

prophylactic therapy should continue for the length of time the individuals remain immunosuppressed.

The incidence of PCP in patients who at least initially received PCP prophylaxis has been evaluated in 2 large studies [19,20]. Table 3 summarizes this information. The overall incidence of PCP was 2.4% in a study from the Brigham and Women's Hospital [19]. In a study from the University of Minnesota, the incidence of PCP was 1.3%, and the survival of patients with PCP was only 37% [20].

Common clinical presentations of PCP include a short duration of symptoms, including fever, dyspnea and cough, bilateral interstitial infiltrates, and a positive bronchoalveolar lavage evaluation in most cases. Not uncommonly, within 24 hours a fever can be followed by the development of extensive bilateral infiltrates. A typical chest x-ray shows *Pneumocystis* as bilateral, diffuse, fluffy, or interstitial infiltrates with a relative central predominance. Uncommon clinical presentations include extrapulmonary pneumocystis (eg, hepatic pneumocystis), nodular granulomatous PCP [21], occasional pleural effusions, normal or minimal chest x-ray findings, and a negative bronchoalveolar lavage results.

### **PCP Prophylaxis**

The CDC guidelines for PCP prophylaxis indicate that all HSCT recipients should receive prophylactic treatment from engraftment for a minimum of 6 months after HSCT. Prophylactic treatment should extend beyond 6 months after HSCT for those who are receiving immunosuppressive therapy (AI) or those who have GVHD (BII). If engraftment is

delayed, PCP prophylaxis can be initiated prior to engraftment (CIII).

Trimethoprim-sulfamethoxazole (TMP-SMX) (AII) is the agent of choice, with alternatives including dapsone (BIII) or aerosolized pentamidine [6]. Because it is less effective for PCP prevention, aerosolized pentamidine is recommended only in those instances in which other agents cannot be tolerated [22]. The CDC guidelines recommend atovaquone as an alternate drug for PCP prophylaxis among dapsone-intolerant patients with HIV [23], but recommendations on its use among HSCT patients have been reserved because data supporting its use are lacking [6].

TMP-SMX is highly efficacious, with rare reports of PCP in patients while on therapy. Side effects are common in HSCT recipients and include rash, myelosuppression, gastrointestinal symptoms, and hemolytic anemia in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency. A double-strength tablet (TMP 160 mg and SMX 800 mg) is recommended 3 times weekly or a single-strength tablet (TMP 80 mg and SMX 400 mg) daily [6,10].

Pentamidine can be administered either in the aerosolized form or intravenously. Aerosolized pentamidine (300 mg once every 3 to 4 weeks via Respigard II nebulizer [Vital Signs, Totowa, NJ]) was associated with a 9.1% incidence of PCP in BMT patients in the Brigham and Women's Hospital study and is associated with a risk of extrapulmonary PCP [19]. Side effects include cough, salivation, and sore throat. Intravenous pentamidine is an alternative prophylactic strategy. Used at a dosage of 5 mg/kg every 4 weeks, intra-

## Atovaquone vs TMP-SMX Prophylaxis for PCP

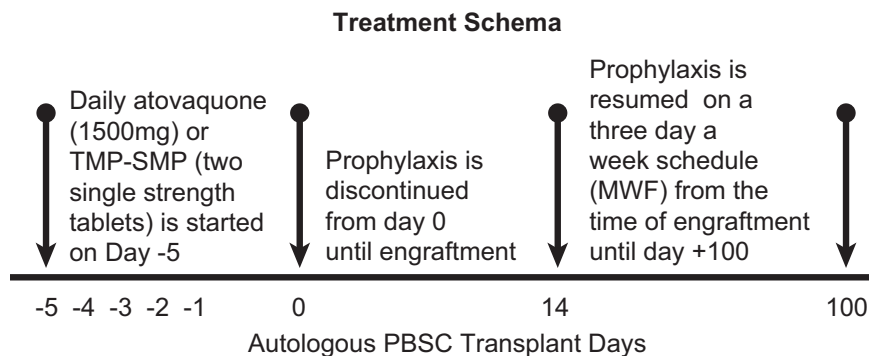


Figure 2. Treatment schema for atovaquone versus TMP-SMX prophylaxis for PCP.

venous pentamidine is effective, with no PCP infections having occurred in HSCT recipients at the Massachusetts General Hospital. PCP infections in solid organ transplant recipients receiving intravenous pentamidine have, however, been reported. Side effects include nausea, hypotension, and paresthesias.

Dapsone (50 mg twice daily or 100 mg once daily), used for PCP prophylaxis, has an approximately 15% cross-reactivity with sulfonamides, which is significant in patients who have a serious TMP-SMX allergy. Dapsone is not recommended in this patient population or in those with G6PD deficiency. Failure rates with dapsone are seen in up to 7.2% of patients [24]. Side effects include GI adverse events, methemoglobinemia, and myelosuppression.

Atovaquone is used for PCP prophylaxis in patients with HIV who are intolerant of dapsone [23]. Its use for PCP prophylaxis in autologous HSCT at the Massachusetts General Hospital has been associated with only a single case of PCP in an autologous transplant recipient who had previously received a heart transplant [19]. Currently, atovaquone is in clinical trials for the prevention of PCP in allogeneic HSCT recipients.

Atovaquone is available in a yellow liquid formulation that, though distasteful to some patients, is easy to ingest. It is generally well

tolerated and side effects, which include rash and GI symptoms, are minimal.

### *Atovaquone versus TMP-SMX Prophylaxis for PCP*

PCP is a common opportunistic pathogen in non-AIDS-related immunocompromised individuals. An estimated 30% of autologous HSCT recipients are intolerant to TMP-SMX therapy. For such individuals, possible alternate therapies include atovaquone, dapsone, or pentamidine. However, because dapsone has a 15% cross-reactivity with sulfonamides, it may not be the agent of choice in patients who are intolerant of TMP-SMX. Pentamidine is associated with a risk of extrapulmonary pneumocystis, and IV pentamidine may require the presence of a central venous catheter, which is associated with a risk of infection. Atovaquone is being evaluated as an alternate agent in HSCT patients.

In a study conducted at the Massachusetts General Hospital in Boston, atovaquone and TMP-SMX prophylaxis for PCP were evaluated in patients receiving autologous HSCT [25]. In a prospective, randomized, open-label trial, 39 patients with various malignancies (primarily breast and ovarian cancer) were randomized to receive 2 single-strength tablets of TMP-SMX or atovaquone oral suspension (1500 mg daily). The TMP-SMX and atovaquone

arms of the study had 19 and 20 patients, respectively. The treatment schema is seen in Figure 2. Treatment was initiated 5 days before and continued until the day before HSCT. Prophylaxis was discontinued at the time of transplantation to avoid the risk of myelosuppression in patients receiving TMP-SMX. After engraftment, prophylaxis was reinitiated 3 times weekly until day 100 after transplantation. Treatment-associated adverse events occurred in 42% of patients administered TMP-SMX and included nausea (1 patient), vomiting (2 patients), neutropenia (2 patients), thrombocytopenia (2 patients), and liver dysfunction (1 patient). Adverse events in these patients led to discontinuation of prophylactic therapy. Treatment-associated adverse effects were not reported in the patient population receiving atovaquone. Although the study was not powered to test efficacy, none of the patients developed PCP during the 100 days of treatment or on subsequent follow-up. Atovaquone is a well-tolerated alternative for PCP prophylaxis in autologous HSCT recipients who are unable to tolerate first-line TMP-SMX treatment.

### *The Atovaquone Experience at M.D. Anderson Cancer Center*

A phase II trial of atovaquone for PCP in allogeneic bone marrow or stem cell transplant recipients intolerant of TMP-SMX has been underway at the M.D. Anderson Cancer Center. The primary end point of the trial is to obtain a survival rate of at least 95% without occurrence of PCP at 180 days. To be eligible, patients had to enroll within 100 days of an allogeneic stem cell or bone marrow graft. In addition, patients were required to have either a known intolerance to TMP-SMX or an inability to receive continued prophylactic treatment with TMP-SMX due to the development of intolerance, defined as an allergic reaction to TMP-SMX with or without associated myelosuppression that necessitated discontinuation of TMP-SMX as determined by attending physicians. Exclusion criteria included active GVHD or any prior history of PCP.



Patients were treated with Mepron suspension (1500 mg PO daily) 5 days prior to transplantation. Treatment was discontinued the day before transplantation and resumed after engraftment (or from the time of discontinuation of TMP-SMX because of intolerance). Treatment was discontinued after withdrawal of the immunosuppression regimen, which was approximately 6 months in most cases. Data on the use of atovaquone in published studies indicate that adverse events include rash, nausea, vomiting, diarrhea, and allergic reaction. Patients who discontinued atovaquone for intolerance were to be evaluated for the development of PCP within the first 6 months posttransplantation. These patients were required to continue PCP prophylaxis according to the current CDC guidelines.

The study was initiated in July 2000. At the time of this writing, 37 patients had been enrolled in the study. Interim analyses indicated that none of the 37 patients had developed PCP. Eight patients (22%) had to discontinue therapy because of intolerance to atovaquone. However, it must be emphasized that these patients developed active GVHD, which may also be associated with GI effects of nausea and vomiting. Thus it is difficult to determine if the adverse events were treatment related or were a consequence of GVHD.

## Summary

Multiple prophylactic regimens are available for PCP and other OIs in HSCT recipients. CDC, IDSA, and ASBMT guidelines present evidence-based recommendations for the prevention of OIs in these patients. However, the choice of regimen should also be based on institutional infection data and individual patient consideration. Prospective trials will be important in determining the optimal strategy for prevention of PCP. Although TMP-SMX is recommended for prophylaxis and treatment of PCP, intolerance and resistance may limit its use. Other recommended drugs include dapsone and pentamidine; however, these are associated with adverse events that may limit their use also. Ato-

vaquone suspension is an alternative to TMP-SMX. It has already received approval for the treatment and prevention of PCP in patients intolerant to TMP-SMX, based on studies conducted in patients with AIDS. Its side effect profile makes it a useful option in the HSCT setting. Compared to TMP-SMX, atovaquone is well tolerated in patients with autologous HSCT. It is currently being evaluated in patients with allogeneic HSCT. Additional clinical experience will indicate whether it may be universally used in autologous and allogeneic HSCT recipients.

## Questions and Answers

**Participant.** The role of atovaquone has been well explained: that it is an agent that may have a place in patients intolerant of TMP-SMX. Can you comment on the role of voriconazole versus fluconazole and valganciclovir versus ganciclovir in this regard?

**Dr. Spitzer.** First, these drugs are not used for PCP prophylaxis. Voriconazole has a much broader antifungal spectrum than fluconazole, including good coverage against *Aspergillus*. Fluconazole has also worked well; but we are beginning to see increasing resistance among non-albicans *Candida*. Infectious disease physicians have been reluctant to recommend voriconazole as routine prophylaxis because it is the best drug that we have, and early indiscriminate use could lead to the emergence of resistant organisms. It should not be used prophylactically but may be administered to high-risk allogeneic transplant recipients. Valganciclovir is essentially identical to ganciclovir; the difference is that as an oral formulation it has better bioavailability than oral ganciclovir. It is therefore easier to deliver in patients who are at risk for CMV infections.

**Participant.** Are there cost or safety issues one should consider?

**Dr. Spitzer.** Voriconazole is well tolerated. Twenty percent of patients show ocular toxicities and visual disturbances, which tend to diminish over time. The cost issue is something we have no information on. Valganciclovir is expensive. But considering it is an oral formulation that can be

administered at home, it is probably going to be cost-effective.

**Dr. Anderlini.** Many of my patients who have been on steroids have shown remarkable responses with voriconazole, mainly for *Aspergillus*. It will be interesting to see how the drug does prophylactically. With respect to valganciclovir, the tablets are fairly large in size and compliance may be a problem because patients have to take several tablets a day. We must remember that these patients are also on other medications. Bioavailability is also an issue with valganciclovir.

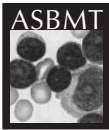
**Participant.** When you addressed influenza prophylaxis, mention was made of chemoprophylaxis. Are there published data on either zanamivir or oseltamivir in the setting of BMT?

**Dr. Spitzer.** I am not aware of any published data with these drugs. I was referring to amantadine and rimantadine. However, the CDC guidelines do not address their use because there are no data concerning BMT patients.

**Participant.** In the present era when monoclonal antibodies are used in different kinds of B-cell neoplasms, we see prolonged periods of CD20 depletion. In this select group of patients, we may see the emergence of PCP after 6 months posttransplantation. We have to be on the lookout for these patients who are pretreated with anti-CD20 antibodies or any other anti-B-cell antibodies.

**Dr. Anderlini.** That is a legitimate concern. I think it is going to be an issue as we use larger doses of agents such as rituximab. This trend exists in some scenarios such as CLL (chronic lymphocytic leukemia) or low-grade lymphomas.

**Dr. Spitzer.** There was a recent report from the Hackensack program that described an unusually high incidence of CMV infections and PML (progressive multifocal leukoencephalopathy) among autologous patients with rituximab as part of their conditioning regimens. As eager as we are to treat B-cell neoplasms and prevent posttransplantation relapses that plague us, we are going to have to look at these patients very carefully for their infection risks.



**Participant:** You talked about the indication for PCP prophylaxis in terms of autologous and allogeneic transplants. In our institution, we are looking at the T-cell numbers from our AIDS patients. This is in terms of patients who are lymphopenic or who exhibit severe aplastic anemia and who are under consideration for HSCT. On another note, because dapsone prophylaxis is associated with the biggest side effect profile, we rarely use it. Myelosuppression is commonly observed, but the PDR has an extensive list of side effects for dapsone.

**Dr. Spitzer:** That's a good point you raised. The CDC guidelines are based on the duration of therapy. I suppose we agree that all HSCT patients should receive PCP prophylaxis. But duration of therapy is very empiric. I'm not certain that anyone has ever looked at T-cell counts posttransplantation to see if there is a correlation with the incidence of infection.

**Dr. Anderlini:** If the correlation between T-cell counts and incidence of infection were validated, it would allow us to stratify our patients. With respect to dapsone, it is also our experience that dapsone is often not well tolerated. I am also concerned about the vicious cycle it propagates. Somebody taking dapsone develops a bizarre skin reaction. The question is: does the patient have GVHD? A biopsy is

performed. And we have to stop the dapsone. This situation may also occur with TMP-SMX. If one can tolerate it, I think using TMP-SMX is the path of least resistance. It provides additional coverage for *Toxoplasma*. It is an inexpensive alternative and has a fairly broad spectrum. The question is: when you cannot take TMP-SMX, what is the best alternative? Physicians have to make their own decisions based on their experience.

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This research summary is presented as a quick guide to an important study that appeared in a recent issue of *Biology of Blood and Marrow Transplantation*. The complete paper, including figures, tables and references, can be found in Volume 8, Number 5, pages 249–256.

## Nonmyeloablative Regimen Preserves “Niches” Allowing for Peripheral Expansion of Donor T-Cells

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### Introduction

Quantitative and qualitative immunologic reconstitution following allogeneic bone marrow transplantation (BMT) has been described, but the mechanisms by which lymphocytes recover and repopulate the immune system remain unknown.

Until recently, no techniques were available to measure thymic production of new T-cells.

This report describes the recovery of T-cells in 5 recipients of cord blood transplants following a nonmyeloablative regimen compared to recovery in adult recipients of cord blood following a myeloablative regimen.

### Methods

#### Patients

Five adult patients without a suitable HLA-matched related or unrelated bone marrow donor underwent mismatched unrelated cord blood transplantation between May 2000 and May 2001 at Duke University Medical Center (DUMC).

#### Conditioning

Conditioning included the use of fludarabine 30 mg/m<sup>2</sup> and cyclophosphamide 500 mg/m<sup>2</sup> daily for 4 days (days –5 to –2) with antithymocyte globulin (ATG) 30 mg/kg per day for 3 days (days –3 to –1). Mismatched unrelated cord blood was infused on day 0. All the grafts were 4/6 HLA matches.

#### Graft-versus-Host Prophylaxis

All patients received cyclosporine (CYA) and methylprednisolone for

graft-versus-host disease (GVHD) prophylaxis.

#### Immunologic Recovery

Immune recovery was analyzed with quantitative and qualitative measures at least every 3 months for the first year after transplantation.

#### TREC Measurement

The concentration of sjTREC DNA in PBMC was measured by quantitative competitive (QC)-PCR following a method previously described.

#### CDR3 Spectratyping

CDR3 spectratyping (immunoscope) was performed every 3 months.

### Results

#### Clinical Recovery

Four of the 5 patients had clear evidence of donor cells. One patient never had any evidence of detectable donor cells. Another patient had minimal (1%) transient detectable donor cells, but these cells did not persist.

Acute GVHD did not occur except in 1 patient (UPN 2611) who developed grade II GVHD and subsequently died of disseminated *Aspergillus flavus* infection. No other patients experienced any unusual or unexpected toxicities.

#### Lymphocyte Subsets

Lymphocyte subset analysis and proliferation assays were performed on all patients who had engraftment. In contrast, the patients who achieved engraftment had a rapid recovery to normal levels within 6 months, at which time all

the cells in the peripheral blood were of donor origin according to microsatellite polymorphism analysis.

#### Immunoscope

Five randomly chosen cord blood units that were not used clinically were tested for their T-cell repertoire. The median number of peaks identified in each of the UCB grafts was 141 (range, 121–185). Spectratyping from the 3 nonmyeloablative recipients demonstrated a remarkable recovery of complexity as early as 3 months following the nonmyeloablative regimen. In contrast, recipients of the ablative regimen demonstrated repertoire skewing between 1 and 2 years after transplantation: the overall number of V $\beta$  families represented and the number of peaks within each family were limited. T-cell repertoires appeared to be more diverse when tested 3 years after transplantation, but they remained substantially skewed in 1 patient.

#### sjTREC Assay

QC-PCR for sjTREC DNA was performed on samples of peripheral blood. TRECs were not detectable prior to 1 year (at 3, 6, and 9 months). By the 1-year mark, both of the surviving patients had sjTRECs above the detection threshold in the peripheral blood (>100 sjTRECs/ $\mu$ g PBMC DNA) with values of 120 and 157 TRECs/ $\mu$ g PBMC DNA. Five randomly chosen cord blood units (not used in any of the patients presented) were also sampled for sjTRECs, and their median was 3913 copies/ $\mu$ g PBMC DNA (standard error, 2291).

## Discussion

This report compares quantitative and qualitative immunological recovery following an unrelated partially matched UCB transplantation in patients receiving a nonmyeloablative or a myeloablative preparatory regimen.

In contrast, the rate of T-cell recovery was markedly different for recipients of UCB transplants who were prepared with a nonmyeloablative regimen. In these patients, the preparatory regimen was the primary difference between the 2 patient populations. The GVHD prophylaxis, ATG dose, and supportive care were identical. The only other major difference was that the patient population receiving the nonmyeloablative regimen had more advanced disease or had a poorer performance status that made them ineligible for conventional ablative UCB transplantation. Although memory T-cells were the dominant subpopulation for the patients receiving the ablative regimen, a rapidly expanding naïve population outnumbered the memory cells in the recipients of the nonmyeloablative regimen. The naïve cells brought the total T-cell count up into the normal range by 1 year after transplantation. In contrast to those receiving the ablative regimens, 2 patients that received nonmyeloablative transplantations had detectable sjTRECs in as early as 1 year after transplantation. The third patient who also engrafted developed acute GVHD, and her recovery was not as robust. The rapid rise of naïve cells and the detectable levels of sjTRECs show that in the recipients of nonmyeloablative regimens, the thymus is functional

early in the posttransplantation course, maturing bone marrow-derived precursor T-cells. Although cord blood itself does contain substantial numbers of new thymic emigrants, sjTRECs remain undetectable through the first year after transplantation. The later increase, therefore, cannot be attributed to adoptive transfer of sjTRECs in the graft but must come from new thymic function. Lastly, although the measured parameters point to a recovering immune system, functional studies are also important. Mitogen responses in the 2 patients with good engraftment have been very robust and not different from those of healthy controls (data not shown). Measurements for cytomegalovirus (CMV) responses using CMV antigen and the FASTImmune assay (CD69 measurement) at the 1-year mark have not yet demonstrated sufficient responses to convincingly demonstrate a robust CMV response. However, CMV reactivation has not been observed in the 3 patients that have had engraftment. Further functional studies are necessary in these patients.

These results suggest that the donor cells only need a niche in which they can proliferate and that the nonmyeloablative regimen does not destroy these niches. Moreover, although the peripheral mechanisms of T-cell expansion are preserved, there is also a suggestion that the central mechanism (thymus) is likewise preserved, in that TREC-positive cells can be detected as early as 1 year following transplantation.

Although the risk of GVHD following cord blood transplantation is lower

than that following matched sibling allogeneic bone marrow or stem cell transplantation, GVHD does occur.

Although not apparent in this analysis, the potential for “holes” in the T-cell repertoire may exist in adult patients. These holes could result from transplantation of a limited number of mature donor T-cells or a subsequent toxic insult to the grafted T-cells (including GVHD prophylaxis). Such voids would be permanent without thymic maturation of new precursor T-cells with novel TCR gene rearrangements. The results of T-cell recovery following the nonmyeloablative regimen suggest that it may be possible to have an excellent outcome with an unrelated mismatched cord blood transplant in adult patients. Patients have a rapid recovery of myeloid cells and platelets and a rapid recovery of T-cells with a complex diversity. The primary difference between the recipients of ablative and nonablative regimens was the extent of physiologic damage caused by the preparatory regimen. When the damage is relatively mild, the donor T-cells are able to expand effectively in the periphery, and the development of new T-cells through the thymus is also accelerated compared to the rate of development in those receiving ablative regimens. Alternatively, the lower incidence of acute GVHD may also play an important role in the preservation of the peripheral and central niches for T-cell development. Future investigation will focus on increasing the chances of engraftment following this nonmyeloablative regimen and expanding these observations.

This research summary is presented as a quick guide to an important study that appeared in a recent issue of *Biology of Blood and Marrow Transplantation*. The complete paper, including figures, tables and references, can be found in Volume 8, Number 7, pages 387–394.

## Response of 443 Patients to Steroids as Primary Therapy for Acute Graft-versus-Host Disease: Comparison of Grading Systems

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### Introduction

Acute graft-versus-host disease (GVHD) is a significant cause of morbidity and mortality after allogeneic hematopoietic stem cell transplantation (HSCT).

To assess the impact of systemic steroids as initial therapy for acute GVHD in the past decade, we retrospectively analyzed the clinical response and survival of 443 HSCT patients, uniformly treated at a single institution from 1990 through 1999 on a protocol of prednisone, 60 mg/m<sup>2</sup> (or methylprednisolone equivalent) for 14 days followed by an 8-week taper.

### Patients and Methods

#### Patients

Clinical and laboratory data were retrieved from the University of Minnesota Blood and Marrow Transplant (BMT) Database, which systematically and prospectively collects data on all consecutive patients undergoing transplantation at our institution. Patients were eligible for the study if they developed within 120 days after HSCT grades II to IV acute GVHD as defined by the Minnesota criteria. Patients with limited (grade I) skin acute GVHD were eligible if there was progression of disease within 7 days or no improvement after 10 days of topical steroid therapy. From January 1990 to December 1999, 1181 patients received an allogeneic HSCT at the University of Minnesota. All transplantation and GVHD protocols were reviewed and approved by the Institutional Review Board. All patients and/or guardians

gave informed consent. Of these 1181 patients, 741 (63%) developed acute GVHD, of which 443 (60%) received systemic steroid therapy as initial therapy and were enrolled in this study.

#### Diagnosis, Staging, and Grading of GVHD

Acute GVHD was diagnosed clinically with histological confirmation whenever possible. Symptoms of acute GVHD were graded by 3 separate grading systems, Minnesota, Consensus, and IBMTR.

Grade of GVHD refers to clinical (not histologic) grade throughout this report.

#### GVHD Therapy

All patients received a daily thrice-divided dose of prednisone 60 mg/m<sup>2</sup> by mouth (PO) (or methylprednisolone intravenous equivalent, 48 mg/m<sup>2</sup>) for 7 consecutive days, then a daily single dose of prednisone for 7 days as initial therapy for acute GVHD. Patients were maintained on therapeutic levels of CSA in 329 patients (74%) or tacrolimus in 15 patients (3%).

#### Measurement of GVHD Response to Prednisone

Response to therapy was evaluated by the attending physician and prospectively recorded in the University of Minnesota BMT Database at treatment days 7, 14, 21, 28, and 42 by determining the GVHD clinical stage score for each time point ( $\pm 3$  days).

#### Statistical Analysis

The major endpoints of this study were response to GVHD therapy at day 28 after treatment and survival. Univariate analysis of response to therapy was performed by Pearson's chi-square test.

### Results

With both the Minnesota and Consensus grading systems, the initial GVHD grades were grade I in 122 patients (28%), grade II in 264 patients (60%), grade III in 50 patients (11%), and grade IV in 7 patients (2%). With the IBMTR severity index, the initial GVHD grades were grade A in 83 patients (19%), grade B in 168 patients (38%), grade C in 181 patients (41%), and grade D in 11 patients (2%). Median time to onset of GVHD from day of HSCT was 27 days (range, 8–94 days). Median time to treatment with prednisone from day of HSCT was 30 days (range, 8–94 days).

Of the 443 patients treated with prednisone, durable response (CR + PR) was observed in 245 patients (55%) by day 28 after initiation of therapy. CR was achieved in 157 patients (35%), PR in 88 patients (20%), and NR in 178 patients (40%). Twenty patients (5%) were unevaluable because of early death but were considered as treatment failures for the purpose of analysis.

Various patient characteristics and transplantation conditions were analyzed for their association with clinical response to prednisone therapy by day 28. Only 9 (31%) of 29 patients given MTX alone as GVHD prophylaxis achieved a response

to steroids compared to 188 (57%) of 329 patients given CSA-containing GVHD prophylaxis, 38 (54%) of 70 recipients of T-cell-depleted grafts, and 10 (67%) of 15 patients given tacrolimus ( $P = .04$ ).

The response to steroid treatment among patients with various combinations of organ involvement was analyzed. The number of organs involved with acute GVHD was not a prognostic indicator of response, as response was observed in 91 (54%) of 170 patients with 1 organ involved with GVHD, 82 (52%) of 157 patients with 2 organs involved, and 72 (62%) of 116 patients with 3 or 4 organs involved ( $P = .23$ ). Patients with lower GI acute GVHD ( $\pm$  other organ involvement) responded less often. Of the 81 patients with lower GI involvement, 34 (42%) achieved CR/PR versus 211 (58%) of 362 patients without lower GI involvement ( $P < .01$ ). The only statistically significant combination of organ involvement was that of lower GI and skin GVHD. Twenty-one (42%) of 50 patients with lower GI and skin acute GVHD obtained CR/PR versus 224 (57%) of 393 patients without this combination ( $P = .04$ ). Organ stage score was not predictive of response to GVHD treatment.

### Chronic GVHD

One year after initiation of steroid therapy, 187 patients had developed chronic GVHD, resulting in a cumulative incidence of 42% (95% confidence interval [CI], 37%-47%). With the Minnesota grading system, chronic GVHD developed in 48 (39%) of the 122 patients (95% CI, 30%-48%) with initial grade I GVHD, 117 (44%) of the 264 patients (95% CI, 37%-51%) with initial grade II GVHD, 21 (42%) of 50 patients with initial grade III GVHD (95% CI, 27%-57%), and 1 (14%) of 7 patients with grade IV GVHD (95% CI, 0%-34%;  $P = .39$ ).

### Infectious Complications

Within the first 100 days after initiation of steroid therapy for GVHD, 182 patients (41%) developed bacterial infections (95% CI, 37%-45%), 14 patients (3%) developed fungal infections (95% CI, 1%-5%), and 96 patients (22%) developed CMV antigenemia (95% CI, 18%-26%). Only 1 patient developed posttransplantation lymphoproliferative disease by day 100 after steroid therapy.

### Survival

In the entire cohort of 443 patients, 234 were alive 1 year after initiation of treatment, with a Kaplan-Meier estimate of 53% (95% CI, 48%-58%) survival at 1 year. Various clinical factors were examined for their association with improved survival. The probability of survival 12 months after administration of steroids was 58% (95% CI, 52%-64%) in related donor recipients, 53% (95% CI, 45%-61%) in HLA-matched URD recipients, and 44% (95% CI, 34%-52%) in HLA-mismatched URD recipients ( $P = .05$ ) (Figure 2). Recipients of T-cell-replete grafts had a higher probability of survival at 1 year than did recipients of T-cell-depleted grafts (55% [95% CI, 50%-60%] versus 40% [95% CI, 29%-51%];  $P = .01$ ).

In Cox regression analysis, the use of a related donor or HLA-matched unrelated graft, CSA as GVHD prophylaxis, younger age at time of HSCT, and lower grade of initial GVHD grade using each grading method were independently associated with greater survival.

### Discussion

This study represents the largest series from a single institution analyzing the effectiveness of steroid therapy as initial therapy for acute GVHD in patients who received HSCT in the 1990s. We observed a response to ther-

apy in 55% of patients and a durable CR in 35% of patients.

Few demographic or clinical factors were statistically predictive of a response to steroid therapy of GVHD. Related donor recipients and HLA-matched URD recipients had similar overall response rates (59% versus 58%) and 1-year survival rates (58% and 53%). In contrast, patients who received HLA-mismatched URD grafts responded less frequently (46%), and their projected 1-year survival rate (44%) was lower. The improved response rates to GVHD therapy in this present study may be due in part to advancements in supportive care. These advancements may also explain our finding of improved response to therapy in patients undergoing transplantation in more recent years.

The number of organs involved in GVHD was not predictive of response to therapy. Patients with lower GI involvement, especially in combination with skin involvement, responded less often.

Recipients of T-cell-replete grafts had a higher probability of survival at 1 year than did recipients of T-cell-depleted grafts.

The Minnesota and IBMTR grading systems better discriminate between initial GVHD grade and survival. Although no GVHD grading system appears superior, the significant discrepancy in assigned grade for a given stage(s) of GVHD is important to note, especially when comparing outcomes of GVHD trials using different GVHD grading systems.

Despite many advances in the past decade in the management of complications related to HSCT, treatment of acute GVHD remains suboptimal. Although a subset of patients may achieve a durable response with steroids, new approaches to GVHD prophylaxis and treatment are needed.

## JOURNAL Watch

**Wright DE, Bowman EP, Wagers AH, et al: Hematopoietic stem cells are uniquely selective in their migratory response to chemokines. *J Exp Med* 195:1145-1154, 2002.**

Despite its importance in the clinical response to stem cell transplantation, little is known about the process by which hematopoietic stem cells (HSCs) migrate into and out of active hematopoietic sites. Previous studies have documented the importance of chemokine-directed migration of other leukocyte subsets. A panel of CC and CXC chemokines was used to study migration of mouse HSCs.

The study model looked at chemotaxis of long- and short-term repopulating HSCs to a panel binding most known chemokine receptors of the CC and CXC families. The HSCs were mobilized using a previously described Cy/G-CSF protocol. In a further study, expression of chemokine receptor mRNA of chemokine receptors on short- and long-term HSCs derived from the bone marrow of untreated mice was studied.

Both the short- and long-term repopulating HSCs migrated only in response to stromal derived factor-1 $\alpha$  (SDF-1 $\alpha$ ), which is the ligand for CXC chemokine receptor 4 (CXCR4). There was no migratory response to any of the other chemokines or to G-CSF. Furthermore, SDF-1 $\alpha$ -induced migration occurred in the absence of non-HSC bone marrow cells. On reverse transcription polymerase chain reaction, HSC were found to express CXCR4. They also expressed mRNA for CCR3 and CCR9, even though they did not migrate to these receptors' ligands. In HSCs derived from the bone marrow of untreated mice, the SDF-1 $\alpha$  response with identical to that of mice treated with Cy/G-CSF.

In this murine model, HSCs migrate only in response to SDF-1 $\alpha$ , not to chemokines signaling through other previously described CC and CXC chemokine receptors. No other leukocyte subset has been reported to have such limited chemotactic responsiveness. This charac-

teristic may play an important role in homing of HSCs to bone marrow and in their maintenance in hematopoietic microenvironments.

**Crippa F, Holmberg L, Carter RA, et al: Infectious complications after autologous CD34-selected peripheral blood stem cell transplantation. *Biol Blood Marrow Transplant* 8:821-829, 2002.**

The use of CD34-selected autologous peripheral blood stem cells (PBSC) transplants is associated with a lower risk of relapse. However, immune reconstitution takes place more slowly, which may place patients at higher risk of infectious complications. One study has reported a higher rate of cytomegalovirus (CMV) infection in patients receiving CD34-selected transplants. Rates of non-CMV infections were assessed in patients receiving CD34-selected vs unselected autologous PBMC transplants.

Two contemporaneous, nonrandomized groups of patients receiving autologous PBSC infusion were retrospectively studied: 32 recipients of CD34-selected PBSCs and 273 receiving unselected infusions. For 100 days after transplantation, the two groups were monitored for the development of fungal, bacterial, and viral infections other than CMV. The two groups received similar infection surveillance and supportive care.

The overall rate of non-CMV infections was 78% in patients receiving CD34-selected PBMCs vs 30% in those receiving unselected infusions. Most of the increase in viral infections reflected higher rates of varicella zoster virus (26% vs 4%) and parainfluenza virus (13% vs 3%) in the CD34-selected group. The rate of bacterial infection was 34% vs 16%. There was no significant difference in the rate of fungal infections.

CD34 selection remained a significant risk factor for viral and all non-CMV infections on multivariate analysis. There was no difference in infection-related mortality.

The use of autologous CD34-selected

PBSC transplants appears to increase the risk of viral and bacterial posttransplant infections. The infection risk is similar to that observed in recipients of allogeneic PBSC transplants, indicating the need for similar infection surveillance and preventive strategies.

**Guglielmi C, Arcese W, Dazzi F, et al: Donor lymphocyte infusion for relapsed chronic myelogenous leukemia: prognostic relevance of the initial cell dose. *Blood* 100:397-405, 2002.**

For patients with chronic myelogenous leukemia (CML) who experience relapse after allogeneic stem cell transplantation, donor lymphocyte infusion (DLI) offers a potentially effective salvage therapy. Questions remain about the timing and dose of DLI; an escalating dose regimen may be used to reduce the severity of graft vs host disease while preserving the graft vs leukemia effect. The outcomes of a large number of patients undergoing DLI were reviewed to assess the prognostic impact of the initial cell dose.

The analysis included 298 patients at 51 European centers undergoing DLI for recurrent CML after first allogeneic stem cell transplantation. Each patient's initial cell dose (ICD) was calculated as mononuclear cells  $\times 10^8/\text{kg}$  transfused at the first infusion. The ICD was 0.20 or less in 98 patients, group A; 0.21 to 2.0 in 107 patients, group B; and greater than 2.0 in 93 patients, group C. The effects of ICD and other variables on patient outcomes were assessed.

Sixty-two percent of patients in group A received additional infusion, compared with 20% of those in group B and 5% in group C. The incidence of graft vs host disease increased with ICD, from 26% in group A, to 53% in group B, to 62% in group C. The incidence of myelosuppression increased as well, from 10% to 23% to 24%. However, response rates were similar across ICD groups: 78% in group A, 73% in group B, and 70% in group C.

Three-year unadjusted survival was 84% in group A, 63% in group B, and 58%

# JOURNAL Watch

Continued from page fifteen

in group C. Failure-free survival was 66% in group A, 57% in group B, and 45% in group C; DLI-related mortality was 5%, 20%, and 22%, respectively. The observed outcome differences remained significant after adjustment for a wide range of patient and treatment variables.

In patients undergoing DLI for relapsed CML, the initial number of mononuclear cells infused has an important impact on key outcomes, including graft vs host disease and survival. The ICD should be no higher than  $0.20 \times 10^8$  cells/kg. This low starting dose will reduce toxicity, although dose escalation will frequently be needed to achieve a response.

**Mutius T, Blokland M, Schrama E, Goulmy E: Generation of minor histocompatibility antigen HA-1-specific cytotoxic T cells restricted by nonself HLA molecules: a potential strategy to treat leukemia after HLA-mismatched stem cell transplantation. Blood 100:547-552, 2002.**

Various approaches have been successfully used to perform stem cell transplantation across HLA mismatches. However, T-cell depletion is commonly performed in this situation, leading to high leukemia relapse rates. A new approach to treating these difficult leukemic relapses—based on generation of hematopoietic system-specific cytotoxic T cells (CTLs) from the stem cell donor—is reported.

The new approach was based on the hematopoietic system-specific minor histocompatibility antigen HA-1, previously shown to induce HLA-A2-restricted CTLs. Peripheral blood mononuclear cells from HLA-A2- individuals served as responder cells, which were stimulated using HLA-A2+ T2 cells pulsed with synthetic HA-1 peptide or HLA-A2+ dendritic cells transduced with HA-1 cDNA. With both approaches, the result was HA-1-specific T cells restricted by "nonself HLA-A2" molecules. These specific CTLs were monitored and enriched with the use of tetrameric HLA-A2/HA-1 peptide complexes.

In alloreactive cultures, three rounds of antigen-specific stimulation yielded up to 7% enrichment of HA-1-specific CTLs and up to an 87% increase in fluorescence-activated cell sorting of tetramer-positive T cells. Further in vitro studies showed that the HA-1-specific CTLs had a specific lytic effect against leukemic and other target cells. The polyclonal CTL cultures were found to contain unwanted allo-HLA-A2-specific CTLs and natural killer cells. Further depletion or selection strategies were carried out to yield clones with exclusive HA-1 specificity.

The results demonstrate the feasibility of generating HA-1-specific CTLs restricted by nonself-HLA-A2 molecules. These cells may be clinically useful for adoptive immunotherapy after HLA-mismatched stem cell transplantation. The authors plan additional studies to assess the generation of specific CTLs using other hematopoietic system-specific antigens.



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