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Introduction

by John R. Wingard, MD
Editor-in-Chief

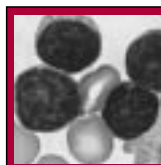
The major challenges facing the clinician caring for patients undergoing hematopoietic cell transplantation are the control of graft-versus host disease and opportunistic infections. With the development of effective strategies to control cytomegalovirus infection, today invasive fungal infections are the leading opportunistic infectious pathogens after HCT. It is, therefore, fitting that the featured topic of this issue addresses the formidable challenges posed by this posttransplantation complication. At the Tandem Transplant Meetings in March, a satellite symposium was held to discuss a variety of issues about fungal infections after allogeneic stem cell transplantation. This symposium was sponsored by Fujisawa Healthcare, through an unrestricted educational grant. The transcript of this symposium is the featured topic in this issue. In the initial presentation, I reviewed the risk factors for invasive fungal infections and the epidemiologic shifts in fungal pathogens that we have witnessed during the past decade. Dr. Brown then discussed pharmacologic and immunologic approaches to antifungal prophylaxis. Dr. Einsele addressed the formidable task of early detection of invasive fungal infection and discussed new rapid diagnostic techniques. In conclusion, Dr. Cagnoni presented recent clinical trial data as to the use of the lipid formulations of amphotericin B and discussed the exciting promise of new triazoles and a new class of antifungal agents, the echinocandins, which are currently in clinical trials. This symposium offers an up-to-date review of the current status and promising new diagnostic and treatment modalities that will likely become available to transplant clinicians in the near future.

2001 TANDEM BMT MEETINGS

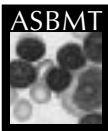
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Be a part of a national organization established to promote education, research, and medical development in the field of blood and marrow transplantation.

PRELIMINARY APPLICATION

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

Associate Membership is open to individuals with an MD or PhD degree who otherwise do not meet the criteria for full membership.

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

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

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ASBMT News

Tandem BMT Meetings Return to Keystone Resort in February 2001

It happens annually: the back-to-back meetings of the American Society for Blood and Marrow Transplantation (ASBMT) and the International Bone Marrow Transplant Registry/Autologous Blood and Marrow Transplant Registry (IBMTR/ABMTR).

In 2001 the meetings will return to Keystone Resort in Colorado:

- ASBMT will meet Thursday-Saturday, Feb. 15-17.
- IBMTR/ABMTR will meet Saturday-Monday, Feb. 17-19.

The tandem meetings allow for less disruption in professional schedules and an opportunity for interaction with a wider circle of colleagues – and with fewer hotel nights and a single airfare. At the 2000 meeting in Anaheim, two-thirds of the 1,422 registrants attended both of the sessions.

Register Online

New this year is online meeting registration, housing reservations, and travel arrangements at the ASBMT Web site www.asbmt.org. All can be arranged in a single visit to the Web site.

Keystone Resort is not unfamiliar to ASBMT members who have attended past national meetings there. However, a newly expanded conference center awaits registrants next February. The Keystone Resort has just opened an \$11.5 million addition, nearly doubling the meeting space. The Tandem BMT Meetings will have exclusive use of the entire facility, billed as “the largest conference venue in the Rocky Mountains.”

Most of the annual meeting sessions and workshops will be held in the mornings and evenings, leaving the afternoons free for skiing and other winter activities. Sleeping accommodations can be selected from more than 1,100 condominiums, studio apartments, and hotel rooms.

Program Sessions

The scientific program will include sessions on:

- Acute myelogenous leukemia
- Allografts as immunotherapy
- Aplastic anemia
- Autoimmune disease
- BMT for solid tumors
- Chronic GVHD biology
- Chronic myelogenous leukemia
- Gene delivery systems
- Genomics
- GVHD classification
- Histocompatibility
- Immune reconstitution and infection
- Late complications
- Lymphoma
- Multiple myeloma
- Natural killer cells
- Pediatric malignancies
- Peripheral stem cell transplantations
- Pre-clinical GVH and GVL
- Sources of stem cells
- Stem cell biology
- Stem cell processing
- Transfusion support
- Transplantations for hemoglobinopathies
- Transplant-related leukemia
- Tumor vaccines

The program chair for the ASBMT Annual Meeting is **Julie Vose, MD**, University of Nebraska Medical Center, Omaha, and co-chairs for the IBMTR/ABMTR Annual Participants' Meeting are **G rard Soci , MD, PhD**, H pital Saint Louis, Paris, and **Donna Reece, MD**, University of Kentucky, Lexington.

ASBMT's Journal Continues an Accelerated Publication Schedule

Throughout the past year, *Biology of Blood and Marrow Transplantation* has maintained an accelerated publication schedule and has moved from a bi-monthly to a monthly frequency.

Among the articles in the recently published Volume 6 are:

Reviews

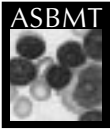
- “A Review of Autologous Hematopoietic Cell Transplantation,” Blume and Thomas, 6:1-12.
- “Varicella-Zoster Virus: Pathogenesis, Immunity and Clinical Management in Hematopoietic Cell Transplant Recipients,” Arvin, 6:219-230.
- “Status of High-Dose Chemotherapy for Breast Cancer,” Nieto et al., 6:476-495.

Biology

- “Complexity of Effector Mechanisms in Cyclosporine-Induced Syngeneic Graft-Versus-Host Disease,” Hess et al., 6:13-24.
- “Humoral Immune Response to Proteins of Human Cytomegalovirus Latency-Associated Transcripts,” Landini et al., 6:100-108.
- “An Immunoablative Regimen of Fludarabine and Cyclophosphamide Prevents Fully MHC-Mismatched Murine Marrow Graft Rejection Independent of GVHD,” Petrus et al., 6:182-189.
- “Real-Time Polymerase Chain Reaction of Immunoglobulin Rearrangements for Quantitative Evaluation of Minimal Residual Disease in Multiple Myeloma,” Ladetto et al., 6:241-253.
- “Cytotoxicity Impaired Transplant Recipients Can Efficiently Resist Major Histocompatibility Complex-Matched Bone Marrow Allografts,” Jones et al., 6:456-464.

Clinical

- “Transplantation of Highly Purified CD34+Thy-1+ Hematopoietic Stem Cells in Patients with Metastatic Breast Cancer,” Negrin et al., 6:262-271.
- “Intentional Induction of Mixed Chimerism and Achievement of Antitumor Responses After Nonmyeloablative Conditioning Therapy and HLA-Matched Donor Bone Marrow Transplantation for Refractory Hematologic Malignancies,” Spitzer et al., 6:309-320.



ASBMT News

continued from previous page

- “Conversion to Full Donor Chimerism Following Donor Lymphocyte Infusion Is Associated With Disease Response in Patients With Multiple Myeloma,” Orsini et al., 6:375-386.
- “Conditional and Unconditional Estimation of Multidimensional Quality of Life Following Hematopoietic Stem Cell Transplantation: A Longitudinal Follow-Up of 415 Patients,” Bush et al. 6:[in press].
- “Immunotherapy with Rituximab During Peripheral Blood Stem Cell Transplant for Non-Hodgkin’s Lymphoma,” Flinn et al., 6:[in press].

ASBMT membership at \$225 per year includes a subscription to the society’s monthly journal, *Biology of Blood and Marrow Transplantation*. A subscription to the journal, separate from ASBMT member, also is \$225.

18 Transplant Centers Gain FAHCT Accreditation

Eighteen centers have recently gained FAHCT accreditation, bringing the number of accredited centers to 30. There are 150 other centers in various stages of application, inspection, or “accreditation pending.”

The Foundation for Accreditation of Hematopoietic Cell Therapy, with 302 trained inspectors, held its first training workshop for cord blood bank inspectors during the annual meeting of the International Society of Hematotherapy and Graft Engineering (ISHAGE) in mid-June in San Diego.

“The process of scheduling inspections, conducting on-site visits, and completing

the Board review has been refined, allowing for a more expedient approval process,” said **Phyllis Warkentin, MD**, chair of the FAHCT Inspection and Accreditation Committee.

The latest facilities to gain voluntary accreditation are listed by categories below.

Accredited for autologous peripheral blood progenitor cell transplantation, including collection and laboratory processing:

- IMPACT Center of Middle Tennessee, Nashville, Tenn.
- Our Lady of the Lake Regional Medical Center, Baton Rouge, La.
- Providence Portland Medical Center, Portland, Ore.
- University Medical Center, Lubbock, Texas

Accredited for autologous peripheral blood progenitor cell collection, marrow and peripheral blood progenitor cell transportation, processing and storage:

- Pacific Northwest Regional Blood Services, Portland, Ore.

Accredited for allogeneic and autologous peripheral blood progenitor cell transplantation, including collection and laboratory processing:

- University of Chicago, Chicago, Ill.

Accredited for allogeneic and autologous marrow and autologous peripheral blood progenitor cell transplantation, including collection and laboratory processing:

- Children’s Hospital of Philadelphia, Philadelphia, Pa.

Accredited for allogeneic and autologous marrow, peripheral blood progenitor cell transplantation, including collection and laboratory processing:

- Cardinal Glennon Children’s Hospital, St. Louis, Mo.
- Christiana Care Health Services, Newark, Del.
- Rush Presbyterian St. Luke’s Medical Center, Chicago, Ill.
- Stanford University Medical Center, Stanford, Calif.
- Texas Transplant Institute, San Antonio, Texas
- University of Minnesota Hospital, Minneapolis, Minn.
- University of Texas, MD Anderson Cancer Center, Houston, Texas
- University of Utah Health Sciences Center, Salt Lake City, Utah
- Wayne State University/Karmanos Cancer Institute, Detroit, Mich.

Accredited for allogeneic and autologous peripheral blood progenitor cell collection, progenitor cell processing, cryopreservation, transport and storage:

- New York Blood Center Clinical Services, Valhalla, N.Y.

Accredited for allogeneic and autologous marrow and peripheral blood progenitor transplantation including cell collection and processing, and allogeneic human cord blood collection, transportation and storage in association with the Civitan Regional Blood Center:

- Shands Hospital at the University of Florida, Gainesville, Fla.

- ◆ 30 Facilities accredited
- ◆ 23 Inspections in progress
- ◆ 73 Facilities inspected and awaiting accreditation
- ◆ 54 Facilities registered and completing checklists

Meeting Report: Fungal Infections After Allogeneic Stem Cell Transplantations (March 29, 2000, Anaheim, California, USA)

Pablo J. Cagnoni,^a Janice Brown,^b Hermann Einsele,^c Andreas H. Groll,^d John R. Wingard^e

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John R. Wingard, MD: Overview of Fungal Infections in Stem Cell Transplantation Patients

Fungal infections are important causes of morbidity and mortality after bone marrow transplantation. With improvements in control of cytomegalovirus (CMV) infection, fungal infection is the leading infection-related cause of death after marrow transplantation today. Crude mortality rates for *Candida* and *Aspergillus*, the 2 major fungal pathogens, are in the range of 70% to 85% for *Candida* and 68% to 95% for *Aspergillus* infections. Regrettably, the outcomes have not improved greatly over the last decade.

A bimodal distribution of occurrence after transplantation for both fungal pathogens has been known for some time. An early peak occurs prior to engraftment, and a later peak occurs typically during the second and third month following transplantation. Deficits in host defenses that predominate early after transplantation and prior to engraftment include neutropenia, damage to the mucosal barrier, and the predominant use of nosocomial procedures such as indwelling venous catheters, all of which can set the stage for invasive fungal infections.

With engraftment, host defenses are restored in this regard but are replaced by yet a different deficit in host defenses, that of cell-mediated immunity. This deficit in cell-mediated immunity can be exacerbated by both the occurrence of graft-versus-host disease (GVHD) and the use of corticosteroids, and it may be more manifest in patients who are recipients of

T cell-depleted transplants. Individuals who experience GVHD continue to have an increase in the frequency of infection over time (Figure 1) [1], whereas recipients of allografts who have no GVHD or auto-transplant recipients are at risk prior to engraftment but subsequently have a very low additional risk.

Obviously, T-cell depletion was used with the hope of diminishing the occurrence of GVHD, thereby reducing the use of corticosteroids. One might have anticipated that this approach would be associated with a lower incidence of invasive fungal infections. Regrettably, that may not have occurred, as illustrated in Figure

2 [1] reflecting the experience at the Fred Hutchinson center, where it was found that the incidence of *Candida* infection over time was substantially higher than with other immunosuppressive regimens. The development of a robust, cell-mediated immunity is important in terms of protection against invasive fungal infection.

Dr. Janice Brown's group from the Stanford University School of Medicine demonstrated in a murine transplantation model that spleen cells from animals immunized against fungal pathogens given after transplantation protected the recipients from serious fungal infection [2]. This is an example of another

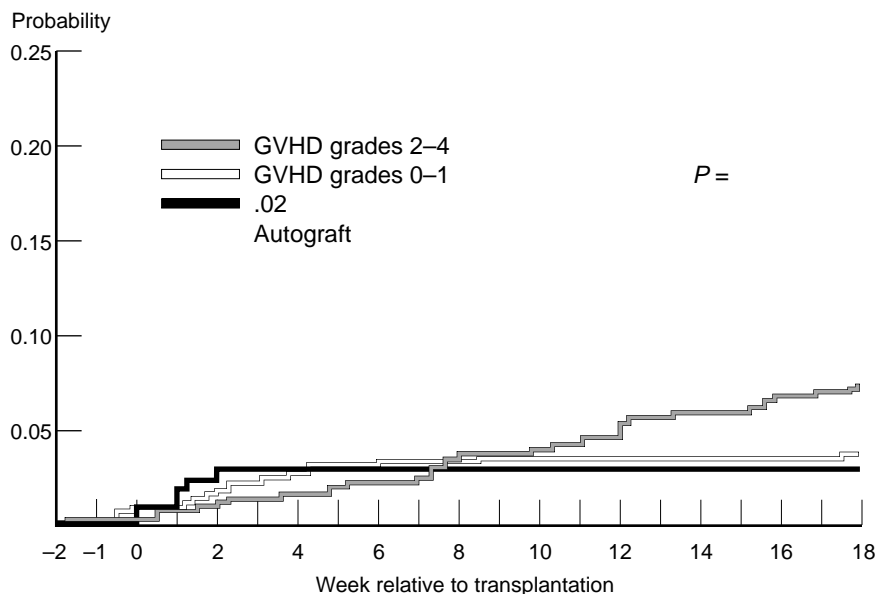


Figure 1. Probability of Aspergillus infection by occurrence of graft-versus-host disease.

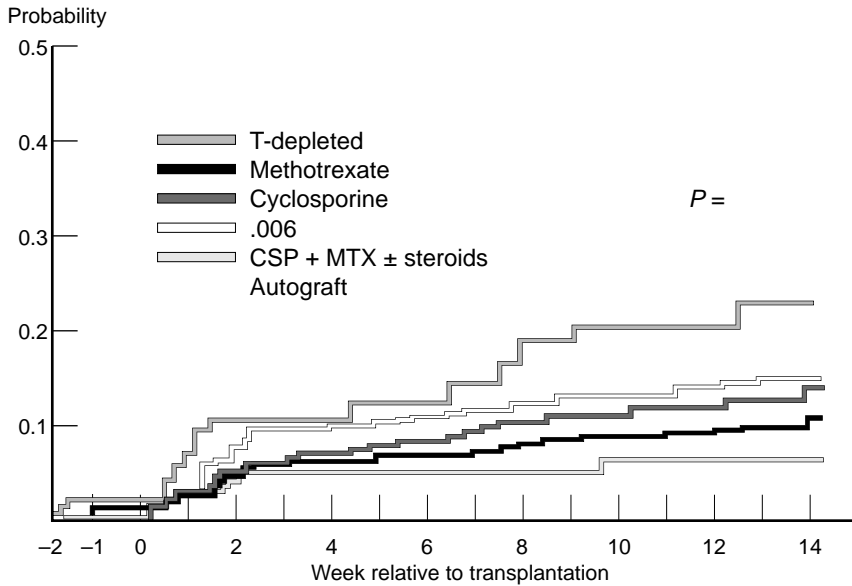


Figure 2. Probability of *Candida* infection by graft-versus-host disease prophylaxis. CS indicates cyclosporine; MTX, methotrexate.

use of adoptive immunotherapy: to protect individuals from potentially life-threatening infection.

As our transplantation practices have changed, so too has the problem associated with invasive fungal infections. Of course, with the pressures of managed care, we are increasingly moving our activities to the outpatient setting. With the advent of hematopoietic growth factors and the recognition of the need to optimize stem cell content in our grafts, we are having shorter periods of neutropenia as well. Combined, these factors reduce that initial peak in the distribution curve of fungal infection early after transplantation and prior to engraftment.

On the other hand, with the introduction of more potent immunosuppressive regimens, (particularly anti-thymocyte globulin or other T-cell monoclonal antibodies), the use of T-cell depletion and the increasing use of alternate donors (either mismatched or matched unrelated donor or cord blood transplants), we are seeing a greater deficit and a more prolonged time to recovery of T-cell function after transplantation, exacerbating the potential risk of fungal infection after engraftment in the subsequent months after transplantations. *Aspergillus*

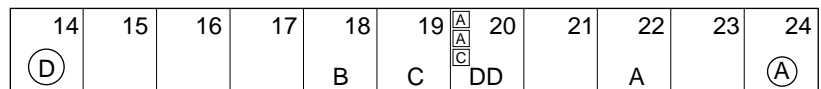
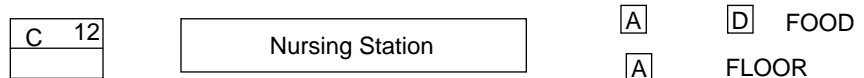
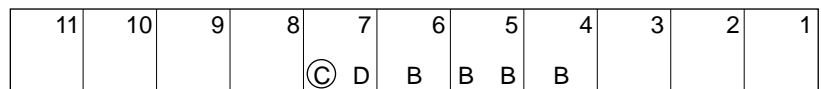
is no longer a problem during neutropenia in the routine transplantation situation, as illustrated by 3 studies from 3 different parts of the planet, Helsinki, Seattle,

and Gainesville [3-5], in which the incidence of *Aspergillus* infection varied between 6% and 15% (yet only a minority occurred during neutropenia, 14%, 31%, and 7%, respectively). Moreover, the average time of onset was between 80 and 100 days; no longer did we see the early peak. In large part, this is because of more rapid engraftment and less vulnerability to the occurrence of infection early after transplantation.

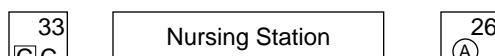
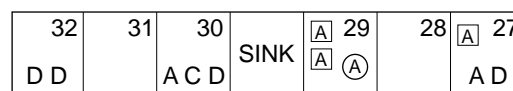
With changes in antimicrobial practices, particularly the widespread use of fluconazole as an antifungal prophylaxis, we are seeing shifts in the pathogens we encounter. Changes have also occurred in the type of invasive *Candida* pathogens encountered. Prior to the 1980s, *Candida albicans* was the focus of concern. In the 1980s, the risk of *Candida tropicalis* became evident. In the 1990s, *glabrata*, *krusei*, *lusitani*, and *parapsilosis* have all plagued patients.

In part this shift in the species of fungal pathogen is related to the introduction of fluconazole. The effects of the widespread use of fluconazole can be illustrated with results from the M.D. Anderson

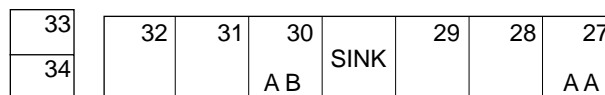
BMT UNIT



ICU 1



ICU 2



- Denotes environmental culture
- Denotes new acquisition
- A-Strain I
- B-Strain II
- C-Strain III
- D-Strain IV

Figure 3. Schematic floor plan of medical intensive care unit (ICU) and bone marrow transplant (BMT) unit. Strains I-IV were most common strain types isolated; numbers 1-24 and 25-34 are patient rooms.

experience [6], in which rates of *Candida* infection over a 4-year span bisected by the introduction of fluconazole in 1990 were evaluated. Rates of infection for 2 years prior and for 2 years following the introduction of fluconazole were provided. The rate of *C. albicans* infections, which had been increasing dramatically prior to fluconazole, dropped dramatically after its introduction. *Candida tropicalis* was declining, and that decline was exacerbated after the introduction of fluconazole. In contrast, there was a leveling off of *Candida glabrata* and an actual increase in the frequency of *Candida krusei* infections over that 4-year span.

Indeed, in a compilation of more than 1600 episodes of *Candida fungemia* in cancer patients [7], it was noted that only half of the *Candida* species were *albicans*. One fourth were *tropicalis*, 8% were *glabrata*, 6% were *parapsilosis*, 4% were *krusei*, and 9% were either not indicated or were other species. No longer is *Candida fungemia* associated primarily with *C. albicans*; these other species are significant as well. Fluconazole is effective against some *Candida* species, but it is not active against others, most notably *Candida krusei*. These organisms are natively resistant to the agent fluconazole.

C. glabrata is different. Some strains are susceptible, and others are resistant. Some initially susceptible strains become resistant over time after exposure to fluconazole. Indeed, many critical care units have seen the recovery of increasing numbers of isolates of *C. glabrata* with higher minimum inhibitory concentrations (MICs) in units in which fluconazole has been widely used. This may yet be an emerging problem that may plague us even to a greater degree in years to come.

Of course, there is little clinically relevant activity of fluconazole against *Aspergillus*, the net effect of which is that there have been, in several instances, well-documented outbreaks of organisms resistant to fluconazole in bone marrow transplantation patients. We reported an outbreak of *krusei* infections in bone marrow transplantation patients and subsequently *C. glabrata*, both at Johns Hopkins [8,9]. Bowden [10] reported an outbreak of *C. parapsilosis* at the Fred

Hutchinson Center. We think of *Candida* as a commensal organism where most infections arise from the flora within the gut, and that indeed the gut is the source of most invasive *Candida* infections. However, *Candida* have DNA polymorphisms just as humans do, and specific strains can be examined and followed over time and place. When DNA polymorphism studies of 2 of these outbreaks were examined, a substantial proportion of these isolates appeared to be the same strain, suggesting both a common source and nosocomial transmission.

How this could have happened can be illustrated by a map of a bone marrow transplantation unit and 2 intensive care units [11]. The bone marrow transplant recipients started off in the bone marrow transplantation unit and were transferred to an intensive care unit (ICU) if they required critical care. Isolates were identified on environmental surfaces and strain typed by colors or letters.

As our patients move, so do those organisms. Of course, they may be transferred by nurses and physicians as well, reminding us yet again that we need to exercise care that we not contribute to the risk of invasive fungal infections. Unfortunately, that is not the end of the story. Other pathogens are emerging, such as *Tricosporin*, *Fusarium*, *Zygomycetes*, *Penicillium*, and *Coccidioides*, azole-resistant *C. albicans*, and the non-*albicans Candida* species that often are more resistant to the azoles.

The drug czar of Florida recently decided to introduce spraying of marijuana with *Fusarium* as a new weapon against growing marijuana, and he planned to pilot test this approach in Gainesville, Florida. Consequently, we in Gainesville can anticipate another fungal problem to be vigilant about. Regrettably, many of these emergent organisms tend to be less susceptible to the agents we have to treat them.

Invasive fungal infections are an important cause of morbidity and mortality after transplantation. Changes in transplantation practices have resulted in shifts in the type of infectious pathogens, and new strategies are clearly needed to help face these new challenges.

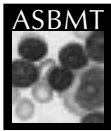
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Janice M. Brown, MD: *Antifungal Prophylaxis Following Allogeneic Hematopoietic Cell Transplantation*

As the rapid evolution of transplantation continues, the term "stem cell" transplantation will become more and more imprecise. Currently, it refers to the transplantation of grafts containing peripheral blood in which mobilized peripheral stem cells have been included with a large number of lymphocytes. There is every indication, however, that this type of allogeneic transplantation will be subject to the same kinds of risks, to a certain extent, as the bone marrow transplantations under discussion.

As Dr. Wingard so eloquently reviewed, the major invasive fungal pathogens include species of *Candida* and *Aspergillus*. I will focus on these fungi and emphasize antifungal prophylaxis with respect to *Aspergillus*. The benefit of strategies outlined here, such as decreasing environmental risks, colonization, nosocomial transmission, and decreasing the use of total parenteral nutrition in antibiotics, have been well established and widely implemented. Numerous



investigators are attempting to corral our nemesis, GVHD, by decreasing the incidence or by decreasing or modifying the use of immunosuppressives necessary to treat or prevent this complication.

Below, two approaches are discussed: the first, a pharmacologic approach to prophylaxis, and the second, an approach based on further understanding of the specific immunodeficiencies we induce in our patients by putting them through the preparative regimen, by modifying the composition of the graft we transplant them with, and by our use of immunotherapy.

With respect to the pharmacologic approach, an intelligent colleague once expressed his skepticism about the concept of antiprophyllaxis by saying it was like trying to salt the roads before it snows. It would probably work to salt the road before it snowed if you had the following conditions: 1) you had the salt; 2) you knew what roads were targeted to be snowed on; 3) the salt would actually stay on the road; 4) it doesn't snow too hard; and 5) you had the appropriate temperature to melt the snow.

Similarly, in an attempt to use a pharmacological approach to antifungal prophylaxis, the following conditions, if met, would lead to a successful antifungal prophylaxis regimen: the correct drug (and moreover a drug that reaches the appropriate tissues—primarily the lung), the appropriate pharmacokinetics of the tissue, a low fungal load, and the appropriate immune response available to help facilitate clearance of the fungus.

The scientific proof that these conditions can be met, as Dr. Wingard explained, can be found in the use of fluconazole as prophylaxis. The results of the 3 largest trials, 2 of which were double-blinded and placebo-controlled prospective studies [1–3], showed that systemic infections could in fact be decreased, as well as fungus-related mortality, and the use of amphotericin B. These studies and others suggest that more than 100 to 200 mg per day or more are required. Again, the risk is that resistant strains may be induced to arise, and there is no clinically significant activity against *Aspergillus*.

We come back to the same problem.

Can one undertake prophylaxis against *Aspergillus* infections? The main drive is the high incidence and the high mortality rate following allogeneic transplantation. Unfortunately, the design of a prophylaxis experiment is fairly cumbersome. Yet, if basic principles are not adhered to, it is difficult to interpret any data. Specifically, a study should be double-blinded and randomized and should use a large sample size. For example, to detect a decrease in fungal infections from 14% to 3% with 80% power will require 330 subjects, and those subjects should not be compared with historical controls. The incidence of *Aspergillus* is not only seasonal in some cases but subject to marked annual variation, so that even at a single institution, the use of historical controls is at best inadequate.

Whether the studies take place at a single center or at many, there should be a similarity in preparative regimens in the use of immunosuppressives (ie, the risks to which the patients are exposed). Below, the currently licensed antifungals for which there have been published studies that included allogeneic patients are reviewed—an easy task because there have been no double-blind, randomized, prospective studies with more than 50 allogeneic patients in which amphotericin B prophylaxis was used.

The three large trials [1–3] all show a trend toward a decrease in systemic infection and a decrease in mortality, although not consistently attributable to a decrease in fungus-related mortality. These trials, however, did not demonstrate a statistical difference in allogeneic recipients. One encouraging common finding was that low doses of amphotericin were tolerated. The study by Perfect et al. [2] was particularly well designed; however, included only recipients of autologous grafts. This study also showed a decrease in mortality unrelated to or not directly attributable to fungal disease.

The 2 other large studies used historic or retrospective analyses. As well outlined in these studies, dramatic changes occurred in the physical nature of the exposures for the patients. The authors of the Wisconsin study [1] report findings that include periods prior and subsequent

to the installation of HEPA filtration. In the case of the O'Donnell study, significant changes in immunosuppressive agents were acknowledged [3]. The studies using a low-dose amphotericin were encouraging, although they were statistically unable to provide definitive proof. Because higher doses of amphotericin B are not well tolerated in allogeneic hematopoietic cell transplant recipients, primarily due to nephrotoxicity, it has not been shown to be a useful prophylactic agent.

However, only limited numbers of studies have been done. Two double-blinded, randomized studies [4,5] were published on the use of liposomal amphotericin used. However, the patients received liposomal amphotericin for a short time only and the studies were underpowered with respect to recipients of allogeneic transplants. In these 2 studies, no statistical differences in systemic infections, mortality, or the use of liposomal amphotericin was observed when 1 mg or 2 mg per kilogram were administered 3 times per week.

Itraconazole solution has been used in 2 studies in an attempt to prevent invasive aspergillosis [6,7]. However, both of these studies included a limited number of patients and only Morganstern et al. included allogeneic patients in their analysis comparing itraconazole solution to a relatively low dose of fluconazole. Their observation of a decrease in overall mortality, in the number of systemic infections, and in amphotericin use is difficult to extrapolate as most centers use a higher dose of fluconazole.

Much additional work needs to be done. Because of the cumbersome nature of trying to accomplish well-designed trials, many investigators have returned to animal models [8]. A normal animal is generally fairly resistant to a high dose of *conidia* whether administered intranasally or intravenously. It is necessary to administer either cyclophosphamide to induce neutropenia or various doses of steroids to render these animals susceptible to invasive disease.

Different agents have been used. Paterson's group [9] used a high dose of fluconazole and actually demonstrated a decrease in mortality and, in the other

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groups that had to use exogenous agents to render the animals susceptible, both amphotericin B lipid complex and human granulocyte colony-stimulating factor were able to decrease mortality from experimental infection. In 1 model of endocarditis from *Aspergillus* in which immunosuppressive agents were not used, amphotericin B and liposomal amphotericin were shown to reduce the incidence of disease and subsequent mortality [10].

In our laboratory, we questioned whether these strategies induced the same degree of immunologic collapse that we witness in our patients, so we devised a model in which the hematopoietic system of a sublethally irradiated mouse is reconstituted with either highly purified stem cells or whole bone marrow. In the prophylaxis studies discussed below, whole bone marrow was administered and then the animals were challenged on the third day following transplantation with *Aspergillus*.

To determine the degree of immunosuppressant, we compared the dose of *conidia* necessary to induce lethal disease with those published in the literature. The dose of *conidia* required to induce lethal disease in animals depends on the route by which the conidia are administered. In normal animals, 100 million *conidia* injected intravenously can induce invasive disease. With the administration of cyclosporine and FK506 or various schedules of glucocorticoids, mice still require a high dose of conidia. In our model, 50 to 100 conidia reliably reproduce lethal disease. If introduced intranasally, we see the same trend, although it is not as profound a difference.

Using this model, we designed a study to determine if prophylactic administration of liposomal amphotericin could protect mice when compared with placebo controls or amphotericin delivered as an attempt to treat infection. In the treatment group, mice received 5 mg/kg of liposomal amphotericin starting 36 hours following inoculation with the conidia. In the prophylaxis group, animals received 4 doses of liposomal amphotericin prior to challenge and then were placed on the same daily schedule as the treatment group. We saw a marked improvement in survival of the

prophylaxis group following intravenous inoculation compared with the treatment group for the animals that received placebo. The results following intranasal administration are even more profound.

A great deal of laboratory and clinical investigation is still required to determine which antifungal agents will be tolerated and what dosing schedule will be efficacious in the prevention of invasive fungal disease. Future attempts to reduce susceptibility to fungal disease should incorporate modulations of the immune response. Given the well documented role of the neutrophil in the host defense against invasive disease, a logical starting point would be to reduce the incidence and duration of neutropenia. Unfortunately, although the administration of growth factors has contributed to the duration of neutropenia, a decrease in fungal infections has not been definitively established.

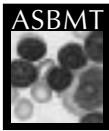
Similarly, although the use of mobilized peripheral blood stem cells does shorten neutropenia, it is unclear whether there will be a decreased incidence of fungal infections. As a result of a fortuitous collaboration with Irving Weisman's group, we decided to examine whether the co-transplantation of novel granulocyte/monocyte progenitors could help protect against invasive aspergillosis. Weisman's group [11] recently published their findings in *Nature*. These oligolineage myeloid progenitors include the common myeloid progenitor (CMP) and, downstream of CMP, the granulocyte-myelomonocytic progenitor (GMP) and the myeloid-erythroid progenitor (MEP). Following differentiation, they propose and have ample evidence to show that common myeloid progenitors subsequently give rise to cells they call granulocyte myelo-monocytic progenitors, which in turn give rise to monocytes and granulocytes. We co-transplanted 10,000 common myeloid progenitors and 20,000 granulocyte-granulocyte-myelomonocytic progenitors with 200 hematopoietic stem cells and challenged these mice with *Aspergillus*, subsequently comparing their survival with mice that received transplants of stem cells alone. Basically, we were able to prove that they were markedly pro-

tected against lethal disease.

Despite these promising findings, it is clear that the elimination of functional neutropenia would not eliminate invasive aspergillosis because the majority of allogeneic transplant recipients are not neutropenic at the time of diagnosis. The specific nature of the immunodeficiencies that result from allogeneic transplantation is an area of active research. In addition to neutrophils, macrophages and platelets have been shown to have activity against *Aspergillus spp.* [12-15] and a growing body of evidence supports an important protective role of acquired immunity [16,17]. Deficiencies in neutrophil, macrophage, and/or lymphocyte function may result following the preparative regimen, as an element of graft-versus-host disease, or due to immunosuppressive agents. The nature of the nonmyeloid antifungal response is still painted in fairly broad strokes at this time. Studies performed over a decade ago using athymic mice were first interpreted as indicating a lack of significant antifungal lymphocyte response. However, there is growing evidence that lymphocytes may play an important role.

Much of the evidence that lymphocytes may have significant antifungal activity comes from elegant work by Cenci, Mehrad, Kurup and others [18-21] that demonstrates a clear association between certain cytokines and susceptibility to lethal aspergillosis. Specifically, interleukin (IL)-4 and IL-10 are associated with an increased susceptibility to disease, and interferon, tumor necrosis factor, and IL-12 production are associated with increased resistance to disease. Exogenous administration of some of these cytokines, especially IL-12, has been shown in murine models of cryptococcal disease, as well as in candidiasis, to offer some degree of protection. Cenci et al. has recently published work showing that in an inhaled model of aspergillosis, exogenous IL-12 additionally confers some degree of protection.

Prophylaxis against *Aspergillus* does appear to be possible. The pharmacologic approach holds a great deal of promise and a growing roster of agents that may be useful if well-tolerated in our patients.



It may be some time before we are able to achieve the appropriate clinical studies to demonstrate this, but we have murine and other animal models at hand to test the principles.

Dr. Einsele will discuss his important findings that demonstrate the potential for identifying patients at risk and may lead to the development of preemptive strategies such as those that have been so successful in the approach to cytomegalovirus. Finally, our growing knowledge about the specific nature of the immunodeficiencies that result following allogeneic transplantation may lead us to alterations in the preparative regimen or approach to graft versus host disease. The scientific rationale exists to support the study of nonmyeloablative preparative regimens, graft modification, and the administration of cytokines in attempts to reduce the morbidity and mortality of invasive fungal infection.

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Hermann Einsele, MD: Early Diagnosis of Fungal Infections

In the last few years, most of us have seen an increase in the incidence of invasive fungal infections, especially of invasive aspergillosis, and a change in epidemiology. At our center, we have seen another group at high risk for developing invasive aspergillosis after allogeneic stem cell transplantation. These are patients that have a history of invasive aspergillosis prior to transplantation. As was seen in a study done by Offner for the European Group for Blood and Marrow Transplantation in EORTC, in a patient that has a previous history of invasive aspergillosis and underwent an allogeneic stem cell transplantation and did not receive antifungal prophylaxis, the risk of recurrence of the invasive aspergillosis is more than 50%. Even if intensified systemic antifungal prophylaxis is used, about 30% of patients will have a relapse of their invasive fungal infection; therefore, this is a group at high risk for invasive fungal infection following allogeneic stem cell transplantation.

Independent of the causative pathogen, eg, *Candida* and *Aspergillus*, if the fungal infection leads to organ involvement, the outcome of the case of a patient with an invasive fungal infection following allogeneic stem cell transplantation is extremely poor, with case fatality rates ranging between 84% and 90%. The only chance to improve the outcome of a patient with fungal infection following allogeneic stem cell transplantation is to detect the infection early. In this setting, the case fatality rate can be reduced to 39%. Therefore, it is extremely important

to make the diagnosis of such an infection as early as possible.

To summarize, we have an increasing incidence of invasive fungal infection and an extremely high mortality rate, not only in the neutropenic but also in the non-neutropenic patients undergoing allogeneic stem cell transplantation; therefore, early initiation of antifungal therapy is essential to improve the outcome of these patients.

Unfortunately, the conventional detection methods are rather insufficient. The aims regarding improvement in the management of patients with invasive aspergillosis are threefold: 1) earlier diagnosis with more sensitive detection methods for the few patients that develop invasive fungal infections during neutropenia; 2) shortening of this period, perhaps by administering additional granulocyte infusions; and 3) in patients developing invasive aspergillosis we have to better define the role of T cells in controlling *Aspergillus* infection and maybe to design adoptive immunotherapy for the future.

Available diagnostic methods include the serological methods (ie, the classical Platelia assay) and the newer pastorex assay, both of which actually detect Galactomannan. We have different nucleic acid amplification techniques, the main one being the polymerase chain reaction (PCR), although new amplification techniques like the NASBA assay might also be available in future for detecting fungal pathogens. Finally, we have radiological techniques.

The serological assays are based on the detection of Galactomannan in the complication of invasive aspergillosis. Galactomannan is secreted by *Aspergillus* and penicillium species and it can be detected by a rat monoclonal antibody. The classic pastorex assay was a latex agglutination assay that detected 15 ng/mL; with the newer assay (the sandwich enzyme-linked immunosorbent assay technique, the Platelia assay), sensitivity can be improved by a factor of 15. Unfortunately, scant published data on this assay exist, and only 1 study showed a sensitivity sufficient for improving the diagnosis of invasive fungal infection.

Regarding radiology, there is the

method of high-resolution computed tomography (CT) scanning. As long as typical lesions like halo signs or crescent signs are found, then this technology is useful. It has been shown that the use of CT when detecting these typical lesions might be able to reduce the time to diagnosis from 7 to 2 days compared with classic radiological techniques. Fortunately, especially in the early posttransplantation period, these classic signs are infrequently seen. Unspecific alterations are often seen with unspecific infiltrates, and it is often difficult to decide whether invasive aspergillosis is already present. As an ideal test to apply to detect fungal infections early, this technique should really fulfill 3 requirements. First, a low number of fungal cells per milliliter of blood has to be detected.

By using quantitative PCR, we have been able to show that the number of fungal DNA copies circulating in the blood, even in patients with invasive aspergillosis, is extremely low; therefore, a high sensitivity of the assay is essential to be able to make an early diagnosis. The other problem is the increasing number of fungal pathogens available, which an ideal test should be able to detect. Because the pathogenicity and antifungal susceptibility vary from species to species, the ideal test should be able not only to detect these various fungal pathogens but also to differentiate and identify them.

We decided to use a PCR assay, and we developed a pan-fungal PCR protocol consisting of 3 parts. In the DNA extraction part, erythrocyte and leukocyte lysis are performed and the DNA is released from the fungal cells by spheroplasting the 3-combinant lyticase. For amplification, we chose a target region that is highly conserved in the primer binding sites, enabling us to amplify a wide range of different fungal pathogens. It is also a multi-copy ribosomal gene, which increases the sensitivity of this PCR assay. The amplification products of these various fungal pathogens are around 500%, and the final detection or the identification of the fungal species is done with genus- or species-specific DNA probes.

The sensitivity of the assay is about 0.1 picogram fungal DNA or 3–10 fungal

cells/mL of blood. The assay is able, because of the conserved region of the primer binding sites, to actually amplify quite a range of different fungal pathogens. Using these species-specific or genus-specific DNA probes, we are able to identify these various fungal pathogens [1].

Inherent in a fungal PCR is a high risk of contamination because of the prevalence of airborne *Candida*, although we found to our surprise that quite a few buffers and lysing enzymes used for DNA extraction are contaminated with fungal DNA because they are produced in situations in which fungal pathogens are prevalent; therefore, we have given up using zymolyase and have switched to recombinant lyticase for releasing fungal DNA [2].

Of course, water is a contamination risk as well; therefore, we use sterile-filtered and ultraviolet-treated water. By using these precautions, the contamination risk can be reduced to an amount also found with viral DNA amplification. It takes about 6 hours to extract the DNA with a classic form of amplification, and it takes 4 hours and another 2 to 6 hours for identification of the fungal pathogen by hybridization or gel electrophoresis [1].

With new methods like automated DNA extraction of clinical specimens and a real-time PCR assay (which allows for additional quantification) [2], we can speed up the process of obtaining the clinical samples considerably. Using PCR and some form of sequencing, this technique may also help us in the future to screen for some of the mutations associated with resistance (eg, azole derivatives).

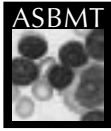
The control testing in our PCR assay and screening of clinical samples sought to determine the specificity of the PCR assay, so we chose more than 200 blood samples from healthy control subjects. Blood samples from neutropenic, noncolonized patients were chosen, and all the blood samples screened were PCR negative. Among the neutropenic colonized patients, 3% were PCR positive. We retrospectively screened patients found to have an invasive fungal infection. When empirical antifungal treatment was introduced, more than 90% of the blood samples were PCR positive for invasive aspergillosis and invasive candidiasis [1].

During the course of antifungal treatment, obviously the number of PCR-positive samples is reduced. When we split up these patients, we found that in patients that responded to antifungal treatment, the percentage of PCR-positive blood samples decreased during the course of antifungal treatment; in patients that did not respond, the percentage of PCR-positive samples remained about the same.

Using these retrospective data, we began a prospective study. Patients undergoing bone marrow transplantation, or peripheral blood stem cell transplantation, were screened by this PCR assay 2 or 4 times per week beginning from the time of conditioning therapy and throughout the time they were in the laminar air flow unit. PCR screening was done until the patient was discharged from the transplantation ward.

These patients were mainly suffering from chronic or acute leukemias. About half received transplants from matched unrelated or a mismatched related donors, and most patients had standard GVHD prophylaxis protocols. Eighteen patients received itraconazole prophylaxis, most of them because of a previous history of an invasive aspergillosis infection, which was proven in 4, probable in 6, and possible in 5 patients. The period of neutropenia following allogeneic stem cell transplantation was less than 500 neutrophils per μL in 15.7 days and less than 100 neutrophils per μL in 12.2 days. Of nearly 1200 samples tested, 169 were PCR positive. Of our patients, 32 of 84 were tested PCR positive at least once. Six tested PCR positive twice, and 12 had more than 2 PCR-positive samples during the posttransplantation period. All 5 patients who had a probable or a proven invasive aspergillosis tested PCR positive.

All 9 patients with a possibly invasive aspergillosis tested PCR positive; among 70 patients that did not develop invasive aspergillosis, 16 were tested PCR positive. A closer look at these 16 patients revealed that 5 had a previous history of invasive aspergillosis, 3 developed invasive aspergillosis in the later posttransplantation period, and 8 of the 70 who tested PCR positive never devel-



oped invasive aspergillosis. After day 40, 3 patients had a proven invasive aspergillosis in the later posttransplantation period (Hebart, 2000).

Of the patients who tested PCR positive, 2 patients who had a possible invasive aspergillosis in the later posttransplantation period again tested PCR positive and 3 of 21 patients tested PCR positive without developing invasive aspergillosis. The sensitivity was 100% and the specificity was between 65% and 74%, depending on when patients with invasive pretransplantation aspergillosis were included. Performing a second test and waiting for 2 positive PCR signals to occur consecutively increased the specificity of the assay (Hebart, 2000).

In the early posttransplantation period, the main risk factor for becoming PCR positive was a previous history of invasive aspergillosis. Risk factors for becoming PCR positive at late posttransplantation, were acute, severe-acute GVHD, and high-dose corticosteroid treatment. Therefore, the PCR assay seems to be suitable for early diagnosis of invasive aspergillosis. It might also help to define patients at risk for developing invasive aspergillosis in the later posttransplantation period and it might be a suitable basis for preemptive therapy.

A risk factor for patients becoming PCR positive in the early posttransplantation period was a previous history of invasive aspergillosis; for the later posttransplantation period, patients with severe acute GVHD receiving high-dose corticosteroid treatment were at high risk. In 1998, a multicenter study was started in Europe to examine PCR-based antifungal treatment with liposomal amphotericin B compared with empirical therapy with lyposomal amphotericin B beginning with a dosage of 3 mg/kg body weight for 3 days and then decreasing to 1 mg/kg body weight (apart from patients who show clinical deterioration).

Another setting in which the PCR screening might be of interest is the screening of bronchoalveolar lavage samples, the rationale being that the documentation of *Aspergillus* species in patients with febrile neutropenia and a pulmonary infiltrate truly makes the diagnosis of an inva-

sive aspergillosis and is clearly an indication to start the patient on antifungal therapy.

With a screening of BAL samples using culture assays, there is a high specificity of 94% to 97% for existing invasive aspergillosis, but the sensitivity is rather low (below 50% in all the studies), and quite a few of the studies indicate that the sensitivity is probably below 20%. Therefore, we hope that screening BAL samples with the PCR technology might improve the sensitivity. Of course, we were also interested in seeing the specificity of this testing.

We received 507 bronchoalveolar lavage samples taken in the University of Essen. The protocol put patients having a bronchoalveolar lavage on day -10, day 1, day 30, day 40, and whenever patients developed pulmonary complications. Surprisingly, only 42 of 507 samples taken from the 134 patients who underwent this protocol were tested PCR positive, 7 of them were the patients who actually developed invasive aspergillosis. Three samples came from 10 patients that were predicted to die from CMV-induced interstitial pneumonia. Another 70 patients developed some form of pneumonia, none of whom tested PCR positive for *Aspergillus* DNA. Finally, 32 bile samples tested PCR positive for *Aspergillus* DNA of 473 BAL specimens taken from patients that did not have pulmonary disease at the time of bronchoalveolar lavage [4].

A closer look at these patients revealed that 7 had tested PCR positive at the time of transplantation, 5 remained PCR positive throughout the whole posttransplantation period, and all developed invasive aspergillosis, some early after transplant. Another 2 patients, at low risk in contrast to the other 5, tested PCR positive but never developed invasive aspergillosis [4].

These results might indicate that a few patients already show colonization in the lower respiratory tract with *Aspergillus* species at the time of stem cell transplantation, which is obviously a high-risk factor for the later development of invasive aspergillosis.

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Pablo J. Cagnoni, MD: New Antifungal Agents

Several of the new antifungal agents that have either recently been or are about to be introduced: liposomal amphotericin B (LAB), amphotericin B colloidal dispersion (ABCD), amphotericin B lipid complex (ABLC), voriconazole, posaconazole, FK-463, MK-0991, liposomal nystatin. The first 3 agents are what have been called lipid preparations of amphotericin B. All of them are on the market and have been available for the last 5 years. One new azole, voriconazole, will be introduced shortly and a second, posaconazole is undergoing phase III and some late phase II testing.

Two agents belong to a new class called echinocandins. An older agent, nystatin, has also been encapsulated in liposomes, and a phase III trial has been completed.

A phase III trial, published several years ago in *Clinical Infectious Diseases* by Dr. Mary White [1] compared a lipid preparation of amphotericin ABCD or amphotericin D colloidal dispersion against conventional amphotericin B for patients with febrile neutropenia. This was a double-blind, multicentered trial for patients with febrile neutropenia who failed intravenous antibiotics. It was a 2-arm trial in which patients either received ABCD at 4 mg/kg per day or conventional amphotericin B at 0.8 mg/kg per day. The endpoints of the study were 2 weeks of therapy or when the fever was identified, when the patient recovered from the neutropenia, or when toxicity led investigators to discontinue the study drug.

Almost 200 patients were enrolled. Successful outcome based on the prior endpoints was equivalent between the 2 arms, 50% for ABCD and 43% for CAB. The incidence of breakthrough fungal infections was equivalent, about 15%.

Documented fungal infections were also similar between the 2 arms, and either defervescence or sustained defervescence was similar between the 2 study drugs. Hence, we can conclude that efficacy was similar overall between these 2 agents.

Renal toxicity was expressed as either a doubling of the baseline creatinine or a drop in the creatinine clearance. Toxicity was significantly lower with ABCD—20% versus 52% with conventional amphotericin B or amphotericin B deoxycholate. Differences were found when subgroups such as children were analyzed: 52% versus 12%, patients that were not taking cyclosporine or tacrolimus, 35% versus 8%, for patients taking cyclosporine or tacrolimus, 68% versus 31%. Significantly less nephrotoxicity from the new agent was seen in this trial.

Toxicity symptoms that occurred in more than 10% of the patients included chills and fever and were not different for ABCD or amphotericin B; the incidences of hypercalcemia and abnormal liver functions were also not different for the 2 drugs; the risk of hypomagnesemia was slightly higher with amphotericin B deoxycholate, although not statistically significant. The incidence of hypoxia during infusion was more common with ABCD.

A second randomized trial involved the use of liposomal amphotericin B (LAB). Approximately a year ago, the *New England Journal of Medicine* published a paper on liposomal amphotericin B versus conventional amphotericin B for empiric treatment in patients with fever and neutropenia [2]. From that study, we have conducted a subset analysis of the allogeneic stem cell transplant recipient. That study enrolled 687 patients, of whom 103 were allogeneic transplant recipients. After extracting the subset of patients from the whole study population, the characteristics of the patients were well-balanced in terms of sex, race, and mean age. The diagnoses were also well-balanced, including acute leukemia, chronic leukemia, lymphoma, myelodysplasia, refractory anemia, and myeloma.

Because one of the endpoints is nephrotoxicity, it is important to point out that patients had similar renal function at

study entry and that the use of fluconazole prophylaxis was slightly higher in the patients on LAB; however, this was not statistically significantly different.

A doubling of the baseline creatinine occurred in about a third of the patients with LAB versus two thirds of the patients with conventional amphotericin B. These results are similar to the numbers referred to previously with ABCD. The number of patients requiring hemodialysis was 1 versus 5. As a result of the doubling of the baseline creatinine, there were a lot more dose reductions of the study drug in the CAB arm compared with liposomal amphotericin B, 60% versus 17%, respectively.

Liver toxicity was not different between the 2 arms. Early reports indicated that some of these lipid products can have more liver toxicity than conventional amphotericin B; however, the overall incidence of liver toxicity or the mean change of different liver function tests—*aspartate aminotransferase*, *alanine aminotransferase*, *bilirubin*, and *alkaline phosphatase*—are significantly different between the arms.

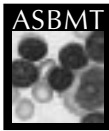
One of the endpoints of the study was to see the overall risk of breakthrough fungal infections. The microbiologically proven infections were all reviewed by an independent reviewer. The LAB arm had only 1 case of proven breakthrough infection (an incidence of 2%) versus 7 infections (an incidence of 14%) in the conventional amphotericin B arm. Many kinds of infections (*Aspergillus*, *Albicans*, non-*albicans*, *Candida*, *C. glabrata*, and 2 nonspecified fungi) were found at different sites of infection, either in the bloodstream or respiratory system. Infections occurred in patients that were or were not taking fluconazole prophylaxis. Two infections occurred in patients on prophylaxis and 5 on patients not taking prophylaxis, the second of which at least could have been prevented with fluconazole. Five of the 7 infections occurred in patients that had their dose of the study drug (in this case conventional amphotericin B) reduced because of toxicity.

The one infection that occurred in the liposomal amphi arm was a *Mucor* infection in a patient not receiving prophylaxis, which would not have prevented this infection anyway.

Another trial involving one or more of these lipid products has been recently submitted for publication by Wingard [3]. His multi-center study compared liposomal amphotericin B versus ABLC for empiric therapy patients with fever and neutropenia. The endpoint of the study was toxicity. This is a relatively small toxicity study with 3 arms: 2 with liposomal amphotericin B at 3 mg/kg per day or 5-mg/kg-per-day and a 5 mg/kg per day arm receiving ABLC. About 80 patients were randomized and received at least 1 dose of study drug. The 3 arms were well balanced in terms of high-risk and low-risk categories, high risk involving most of the allogeneic transplantations. Patients' characteristics in terms of diagnosis and type of transplant received were also well balanced across the 3 arms, with no major differences. The overall success rate was similar across the arms: 40% and 42% for the 2 liposomal amphotericin arms versus 33% for ABLC.

One difference between the arms was the incidence of drug discontinuation from toxicity, 12% and 13% in the liposomal arms and 32% in the ABLC arm. Again, this was a double-blind randomized trial in which physicians were able to discontinue the agent if they thought that too much toxicity had occurred. The reasons for that discontinuation include chills and fever during the infusion and an increase in creatinine. The latter is significantly lower with liposomal amphotericin. The incidence of hypoxia was lower with liposomal amphotericin B. Nephrotoxicity was one of the endpoints, determined as a doubling of the baseline creatinine level. Forty-two percent of the patients on ABLC doubled their creatinine versus about 15% of liposomal amphotericin B patients. However, a doubling of baseline creatinine is not always clinically meaningful.

About 18% of the patients in the ABLC group have creatinine levels of more than 2.5 mg/dL versus 5.5% for liposomal amphotericin patients. About 13% in the ABLC group had creatinine levels greater than 3 mg/dL versus 4.2% for the liposomal amphotericin B patients. Liver toxicity was not different across the arms.



One agent that has recently undergone complete phase III testing—and should be launched soon—is a new triazole, voriconazole. The in vitro and animal data are promising. In vitro data have shown that voriconazole is more active than itraconazole against certain molds, particularly *Fusarium*. Also very encouraging is that voriconazole is active against amphotericin B-resistant *Aspergillus*, and it is certainly active against azole-resistant *Candida*.

Animal models have shown voriconazole to be effective against azole-resistant *Candida* species as well. Voriconazole can be given orally or intravenously, which might represent an advantage in patients that need to take a drug for long periods of time.

The echinocandins are a new class of agents that we will hear more about in the next few years. These drugs have a different mechanism of action compared with some of the agents we have been discussing. They inhibit beta-(1,3)-D-glucan synthesis in the fungal cell wall (amphotericin acts on the cell membrane). Echinocandins have a broad spectrum of activity, being active against azole-resistant *Candida* and fungistatic against *Aspergillus*; however, they are inactive against *Fusarium*, *Trichosporon*, and *Cryptococcus*. At least 3 other agents are in clinical development: MK-0991, LY303366, and F463.

The efficacy of FK-463 has been confirmed in patients with HIV-associated candidiasis. A US maximum tolerated dose (MTD) study of stem cell transplant recipients was recently completed and has been submitted for publication. Our center participated along with the University of Illinois, Duke University, and the University of Florida. The study increased doses of FK-463 up to 200 mg a day. No toxicity associated with FK-463 was seen after this dosage, and the study was stopped at that time.

FK-463 is also active against *pneumocystis carinii*, which might make it an interesting agent for prophylaxis in patients at risk for both fungal infections and pneumocystis. No antagonism exists with amphotericin B. The MTD study was presented by John Hiemenz at the last ICAAC meeting last September involving stem cell transplant recipients. The initial dosage of 12.5 mg per day, given from the day of transplantation

until engraftment was increased up to 200 mg/day. This study addressed the early risk for aspergillosis after a transplantation, and although that might not be where the biggest problems are, the study was designed to address the risk of fungal infection during the neutropenic period. It was a 2-arm study in which 2 patients per dose level received fluconazole alone and 8 patients received fluconazole and FK-463.

It was not considered ethical to do a placebo arm for reasons that are obvious when one looks at the data with fluconazole. Twelve control patients and 62 active patients took part. The active patients received FK and fluconazole, the control patients fluconazole alone. There was no difference in the incidence of mucositis, fever, thrombocytopenia, rashes, headaches, diarrhea, or hypocalcemia. Serum creatinine, which is frequently a problem in allogeneic transplantation patients particularly, was not different between the 2 groups.

Mean creatinine levels at baseline and at the end of therapy were not different for the control group, and there were no major differences in any of the dose levels. Serious (grade 3 or 4) adverse events that the investigators reported as possibly related to one of the study drugs include 1 case of a patient that received fluconazole alone and 3 other cases—atrial fibrillation, hypocalcemia, and a rash—in patients who received FK-463 plus fluconazole at 3 different dose levels. Five patients died in the study: 1 control patient died of bacteremia, and 4 patients who received FK-463 plus fluconazole died of one of the following complications: veno-occlusive disease, heart failure, fungal pneumonia, or cardiac arrest. Because the day of death is at least 7 days away from the last dose of the study drug in all of the patients, it is unlikely, although possible, that the drug contributed to the cause of death in any of these patients.

Ongoing studies with echinocandins include a phase III study of FK-463 versus fluconazole for antifungal prophylaxis in stem cell transplantation patients. Another phase III study that has recently begun is MK-0991 versus liposomal amphotericin for the empirical treatment

of patients with febrile neutropenia who have undergone a stem cell transplantation. Two phase II FK-463 studies on candidiasis and aspergillosis are also ongoing.

A large, randomized trial has been recently reported of another new drug, liposomal nystatin. Nystatin has been around for a long time, mainly for topical use. Not long ago, investigators were able to encapsulate it in liposomes, therefore reducing toxicity and making systemic administration possible. Early in vitro data, most of which has been generated by Dr. Walsh's laboratory and Dr. Groll [4], show that liposomal nystatin is active against *albicans* and non-*albicans Candida* species. It showed its efficacy as well in different animal models, such as neutropenic rabbits with candidiasis and neutropenic mice with aspergillosis. On the basis of some of these data, a large, randomized trial was conducted in which we participated.

Almost 540 patients with febrile neutropenia were enrolled. There were 2 arms: liposomal nystatin at 2 mg/kg per day or conventional amphotericin B at 0.6 to 0.8 mg/kg per day. The 2 groups were well balanced in terms of Apache score, transplant history, antifungal use, or use of different antibiotics. The authors used parameters of efficacy, including survival for 3 to 5 days posttherapy, no breakthrough infections, and no discontinuation because of toxicity. Overall success was seen in about 36% of patients with liposomal nystatin and 39% of the patients with conventional amphotericin B. However, the liposomal nystatin arm had a lower incidence of increased creatinine, a lower incidence of increased blood urea nitrogen, and a lower incidence of hypocalcemia.

To summarize, several agents are close to being launched, and many more are undergoing study. All are likely to make our future choices of antifungal agents more difficult, but hopefully we will have more efficacious agents with which to treat our patients.

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Andreas H. Groll, MD: New Directions in Antifungal Therapy Research

Invasive opportunistic fungal infections have emerged as important causes for morbidity and mortality in immunocompromised patients. Recent epidemiological trends indicate a shift toward infections by *Aspergillus* species, non-albicans *Candida* species, and previously uncommon fungi, and that all have limited susceptibility to current antifungal therapies [1–7].

For many years, the treatment of invasive fungal infections consisted of amphotericin B deoxycholate (D-AmB) with or without the addition of 5-fluorocytosine (5-FC). Therapeutic options emerged only with the clinical development of fluconazole and itraconazole in the late 1980s. The past 10 years, however, have witnessed a major expansion in antifungal drug research. These efforts are reflected in the introduction of the lipid formulations of amphotericin B and the advanced clinical development of improved triazoles, novel echinocandin derivatives and a multilamellar liposomal formulation of nystatin [8].

Nevertheless, despite these advances, each of the currently approved and investigational antifungal agents has its specific limitations. Responses to treatment remain unsatisfactory overall, and there is a continuing urgent need for improved approaches to treatment and prevention.

Current avenues to advance the prevention and treatment of invasive fungal infections include the discovery of novel targets and therapeutics, the modification of existing compounds, the investigation of the mechanisms and significance of antifungal drug resistance, establishment of predictive in vitro susceptibility testing methods, understanding of the pharmacokinetic and pharmacodynamic relationships of antifungal drugs, exploration of combination therapies, restoration of host defenses, and incorporation of surrogate markers in therapeutic algorithms. Most important of all, however, is the rational design of clinical trials that will ensure the implementation of new knowledge into clinical practice.

Existing classes or compounds may be improved by a systematic analysis of their structure–activity relationship and optimization in their molecular structure which may lead to enhanced potency and target selectivity, expansion of the antifungal spectrum, or improved pharmacokinetics. A prime example of this approach are the novel triazoles, posaconazole and voriconazole, that have been designed based on the structure of itraconazole and fluconazole, respectively [9].

The utility of existing compounds may also be enhanced by the development of novel carriers that improve the pharmacokinetics of a drug. This is exemplified by the oral cyclodextrin formulation of itraconazole that achieves significantly better gastrointestinal absorption compared to the capsule formulation [Barone 98]. Novel carriers can also modulate the pharmacodynamics of a drug as in the case with the lipid formulations of amphotericin B that are all associated with less nephrotoxicity than conventional amphotericin B [10].

The pathways to novel targets and the development of entirely new antifungal therapeutics is considerably more difficult and paved with failed projects. One reason for the slow progress in this area is that, owing to their eukaryotic nature, fungal cells have a restricted set of specific targets that do not overlap with their mammalian counterparts, and they carry the potential of mechanism-based toxicity. Whole-genome sequencing, bioinformatics, and advances in proteomics and stereochemistry hold promise for an accelerated identification of specific targets and the development of selective antifungal compounds [12]. Nevertheless, compared with the realm of antibacterial chemotherapy, the antifungal armamentarium is likely to remain limited for the foreseeable future. It is not only for this reason that continuing efforts are needed to further develop the foundations of current approaches to the treatment of invasive fungal infections.

Because of their eukaryotic nature, genetic exchange mechanisms, which majorly contribute to the emergence and spread of antimicrobial resistance in pathogenic bacteria, are largely unknown in fungi. At present, the encounter of resistance to anti-

fungal drugs is essentially limited to the following two situations [13]: first, the primary encounter of a naturally resistant species, as exemplified by *Trichosporon beigelii* or *Pseudallescheria boydii*, which are inherently resistant to the fungicidal activity of amphotericin B; and secondly, the selection of inherently resistant species during antifungal therapy, as exemplified by breakthrough infections with *Candida krusei* or *Candida glabrata* during systemic prophylaxis with fluconazole [8]. In contrast, cumulative molecular events that lead to progressively decreased susceptibility or stable resistance during exposure to current azoles are rarely encountered in patients and have only been anecdotally reported in conjunction with HIV-associated oropharyngeal candidiasis and longstanding exposure to azoles [14,15]. However, along with the widespread use of antifungal azoles in medicine and agriculture, selection and spread of azole-resistant *Candida* spp. appear to be inevitable. To meet this challenge, a better understanding of the molecular mechanisms of antifungal drug resistance is required. During the past few years, alterations at the target binding site, increased target expression, and the presence of inducible efflux pumps have been identified as mechanisms of azole resistance and may offer targets for intervention. Comparatively little is known about polyene resistance, but changes in the composition of the fungal cell wall and in the sterol chemistry of the cell membrane have been described in amphotericin B-resistant fungi [16].

Establishing reproducible and predictive in vitro methods for assessing antimicrobial susceptibility is an extremely important tool for the identification of resistant organisms and for the optimal selection of antimicrobial agents. The experience in the realm of antibacterial chemotherapy indicates superior outcomes for therapies that are guided by the results of in vitro susceptibility testing as opposed to a merely species-based therapy [17]. A standardized method is now available for testing the in vitro susceptibility of yeasts to current antifungal agents [18], and a similar method has been proposed for filamentous fungi [19]. However, correlation of in vitro susceptibility with antifungal activity in vivo

remains difficult to establish [20]. This is in part related to ongoing methodological problems particularly with the definition of the optimal medium and assay endpoints, but also to the circumstance that host- and disease-related factors play a prominent role in the outcome of most invasive fungal infections.

It is largely due to the importance of host- and disease-related factors that animal models are so pivotal for the evaluation of new antifungal therapeutics [21–23]. In contrast to the clinical setting, animal models of invasive fungal infections allow for a high degree of control of covariates and true outcome measurement. Although screening models are indispensable to provide the proof of principle for in vivo efficacy, discriminative infection models aim at simulating the underlying deficiency in host defenses and the pathogenesis and treatment of human mycoses in order to establish a scientific foundation for clinical studies. Notably, for infections by uncommon fungal pathogens, insights derived from infection models can serve as the sole source for evidence-based treatment decisions in individual patients [24,25].

Highly discriminative persistently neutropenic rabbit models of disseminated candidiasis [22,26] and invasive pulmonary aspergillosis [27,28] have been particularly useful in providing a platform for the evaluation of antifungal therapeutics in granulocytopenic patients, and similar models have generated important information on the pathogenesis and therapy of some of the emerging fungal pathogens [29]. The unique features of these models include the surgical placement of a central venous catheter for secure, repeatable, and atraumatic access to the bloodstream, the induction and maintenance of profound neutropenia (<100 granulocytes/ μ L), the administration of broad spectrum antibiotics for prevention of invasive bacterial infections, and the utilization of natural routes of infection for inoculation. The efficacy of antifungal interventions is evaluated by a composite of endpoints that includes survival probability, the clearance of relevant target tissues from the infecting organism and, in the case of invasive pulmonary

aspergillosis, the number of hemorrhagic infarcts and lung weight as parameters of organism-mediated tissue injury. The models are also useful in assessing the potential value of computer-assisted imaging and surrogate markers such as metabolites, antigens, or PCR products for early diagnosis and monitoring of invasive fungal infections [30,31]. Finally, as exemplified in the preclinical evaluation of the lipid formulations of amphotericin B, integration of endpoints of laboratory toxicity allows for detection of important differences in the safety of antifungal agents [27]. However, it is clear that even the most sophisticated model cannot simulate the variability in immune status, underlying disease processes, and infecting fungal strains in patients and replace clinical studies.

One of the principal aims of antimicrobial drug therapy is the characterization of the relationships between dosage, drug concentrations in the body, the in vitro susceptibility of the microorganism, and drug effects. Understanding these pharmacokinetic–pharmacodynamic relationships provides important knowledge of a drug’s mode of action and can be instrumental in setting susceptibility breakpoints and guiding optimal dosing regimens [328]. For antifungal drugs, the assessment of pharmacokinetic and pharmacodynamic relationships relies on well-controlled infection models that provide true endpoints. Such models allow for the exploration of the relationships of pharmacodynamic parameters such as C_{max}/MIC , AUC/MIC (and the time in which plasma concentrations stay above the MIC), with antifungal efficacy in dose-fractionating studies, and for the description of the magnitude of these parameters that is required for antifungal efficacy.

Current approaches to treating most invasive mycoses are based on dosages and dosage schedules that have been empirically derived over time. Pharmacokinetic–pharmacodynamic relationships are only beginning to be explored. Recent experimental studies suggest that the antifungal effects of D-AmB against *Candida* spp. are related to peak concentrations [33,34], and those of fluconazole to the time during the dosing interval that is

spent above the MIC [35]. Incorporation of pharmacodynamic endpoints into clinical studies, however, has not been undertaken, but remains an important goal.

The availability of drugs with different molecular targets has opened new avenues for exploring combination therapies of 2 or even 3 drugs. The obvious aims of combination therapies are to broaden the antifungal spectrum, to decrease the selection of resistant clones, to reduce treatment-associated toxicity, and, most importantly, to improve overall antifungal efficacy. The paradigm for this approach is the combination of D-AmB and 5-FC, that shows synergistic activity against *Cr. neoformans* in vitro and in animal models which also translates into superior outcome in patients with cryptococcal meningoen- cephalitis [36,37]. However, careful preclinical evaluation of combination therapies in vitro and in animals is warranted before their utilization in patients. This is exemplified by the observation of a drug- and fungus-specific antagonism between D-AmB and the antifungal azoles, that has been consistently noted mainly with the lipophilic azoles and with *Candida* and *Aspergillus* spp. [38,39]. Unless evaluated in controlled clinical studies, the combination of D-AmB with any azole can therefore not be recommended [40].

Restoration of host defenses is a goal of paramount importance in the quest for improved therapies for opportunistic mycoses. A considerable body of preclinical in vitro and in vivo data has now accumulated that shows that cytokines, effector cells, and antifungal drugs can work synergistically to oppose fungal growth [41]. Of particular interest for the setting of allogeneic stem cell transplantation may be the observation, that granulocyte/macrophage colony-stimulating factor can restore corticosteroid-induced impairment of phagocytic function without interfering with suppressed lymphocyte function [42]. In addition, there is experimental evidence that T cell– helper–dependent immunity plays an important role in host defenses against invasive aspergillosis. This may explain the surge of invasive pulmonary aspergillosis in allogeneic transplant recipients after engraftment, even in the absence of corticosteroid

therapy. Cytokines and anti-cytokines that promote this pathway (i.e., interferon- γ , interleukin [IL]-12 and anti-IL-4) may be protective in vivo and act in cooperation with antifungal drugs [43,44].

Investigating new antifungal therapeutics is true translational research. In an iterative process, questions generated from in vitro experiments or clinical observations are tested in discriminative animal models to create a solid scientific platform for clinical studies. During the past decade, major progress has been made in defining paradigms for antifungal intervention and in designing and implementing clinical trials investigating new antifungal agents targeted against opportunistic mycoses [45]. However, many strides remain to be made, including but not limited to the prevention and management of invasive aspergillosis, disseminated candidiasis, and infections by and increasingly large pool of emerging fungi.

Cognizant of past and present epidemiological trends, invasive fungal infections will likely remain a frequent and important complication in immunocompromised patients. Identification of high-risk patient populations, improvements in early diagnosis, an expanded drug arsenal, elucidation of resistance mechanisms, integration of pharmacokinetic and pharmacodynamic relationships, multiple-drug regimens, and adjuvant immunotherapies offer hope for further substantial progress in prevention and treatment.

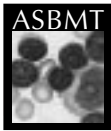
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Questions and Answers

Participant. I have a question for Dr. Brown related to growing concerns regarding azole-resistant *Candida* species and the use of prophylaxis in the bone marrow transplantation setting. Are there any solid data suggesting that the use of pharmacologic prophylaxis decreased the requirement or the use of empiric amphotericin B administration rate in the setting of acute posttransplant conditions?

Dr. Brown. Actually, the 3 large trials did indicate that there is a decrease in the use of empiric amphotericin B, with respect to resistance, that seems to be institution-dependent at this point. Some institutions are reporting, as Dr. Wingard noted, an increase in the appearance of intrinsically resistant species such as *Candida krusei*. Other large centers that have been using fluconazole do not necessarily see that trend as of yet. Part of that might be surveillance deficiencies. Maybe some centers are not as well scrutinized as Dr. Wingard's. Again, it's not necessarily pre-



dictable, but it is a concern.

Participant. In terms of duration of therapy for patients who have documented organ involvement with *Aspergillus*, are you treating the fungal infection until all symptoms are gone on a CAT scan, for instance, or are you treating to some defined dose?

Dr. Einsele. In patients that have invasive aspergillosis, I treat until resolution in the CAT scan, but if the patient continues to be immunocom-

promised, I continue. You could define a secondary prophylaxis, but I don't stop all therapy just when the CAT scan findings resolve. That seems to be a consensus. So you continue therapy basically as long as the patient continues to be immunocompromised.

Participant. Could you comment on the itraconazole suspension, in terms of how effective it is for *Aspergillus*?

Dr. Einsele. I don't think good solid data exist regarding its efficacy. In

patients with invasive aspergillosis, I use amphotericin B as first-line therapy and itraconazole in either oral suspension or intravenously, now that it's available; particularly oral suspension for long-term secondary prophylaxis.

Dr. Wingard. Unfortunately, in the prophylaxis study, the rates of invasive *Aspergillus* infections were low in both the control and the treatment arms. There were actually slightly more frequent but not statistically significant

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.

Apostolidis J, Gupta RK, Grenzeliis D, et al: High-dose therapy with autologous bone marrow support as consolidation of remission in follicular lymphoma: long-term clinical and molecular follow-up. *J Clin Oncol* 18:527-536, 2000.

The 5-year outcomes of patients receiving high-dose therapy (HDT) for follicular lymphoma are reported, including the prognostic significance of polymerase chain reaction (PCR)-demonstrated molecular remission. The experience included 99 patients, median age 45 years, in second or later complete remission or in good partial remission. In addition to cyclophosphamide and total body irradiation, HDT included collection and reinfusion of bone marrow that was treated in vitro with anti-B-cell antibodies and complement. To assess molecular remission at follow-up, a PCR was performed to detect Bcl-2/IgH rearrangement in bone marrow.

At a median follow-up of 5.5 years, 65 patients were alive, and 49 of these were free of treatment failure. Seven of ten late treatment-related deaths were caused by secondary myelodysplasia or secondary acute myeloblastic leukemia. Overall, 12% of patients had one of these complications. Five-year Kaplan-Meier freedom from recurrence was 63%, and the 5-year survival rate was 69%. Patients who lacked the Bcl-2/IgH rearrangement at baseline had a better prognosis with a hazard ratio of 0.39. Prognosis was also improved for those with 3 or fewer previous episodes of treatment. Among patients who did have the Bcl-2/IgH rearrangement, absence of this rearrangement on follow-up PCR carried a reduced risk of recurrence and death. However, the PCR findings of the reinfused bone marrow were not significantly related to patient outcome.

Intensive therapy consisting of HDT plus autologous bone marrow support improves freedom from recurrence in patients with follicular lymphoma. However, no significant improvement in survival is noted so far, compared with historical controls. Elimination of the Bcl-2/IgH rearrangement on follow-up PCR is a good prognostic sign. High-dose therapy carries a significant risk of secondary myelodysplasia, which can cause late treatment-related death.

Dickinson AM, Sviland L, Wang XN, et al: Predicting graft-versus-host disease in HLA-identical bone marrow transplants: a comparison of T-cell frequency analysis and a human skin explant model. *Transplantation* 66:857-863, 1998.

An in vitro skin explant model and T-cell frequency analysis were assessed for their ability to predict the development of GVHD in recipients of HLA-identical sibling bone marrow. A combined limiting dilution assay was used to calculate the frequencies of host-reactive cytotoxic and helper T lymphocyte precursors (HTLp and CTLp, respectively). In addition, a skin explant model was used to assess the presence of a graft-versus-host reaction (GVHR). The incidence of clinical GVHD was compared with the HTLp/CTLp frequency and with the presence of an in vitro GVHR.

Of 13 patients with a positive GVHR, 10 developed GVHD, compared with just 2 of 9 patients without such a response. All 18 patient-donor pairs studied had low frequencies of HTLp, none higher than 1:100,000/ However, one half of the recipients went on to develop clinical grade II or higher GVHD. There was no relationship between the CTLp frequency and the incidence of GVHD.

The frequency of host-reactive HTLp or CTLp is not a useful predictor of GVHD among recipients of HLA-identical sibling bone marrow. However, a positive

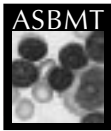
GVHR in the skin explant model does reflect the risk of GVHD, probably as an indicator of disparities in the minor histocompatibility antigen. The use of this assay for typing of patient/donor pairs warrants further study, and may help to improve GVHD prophylaxis.

Domen J, Cheshier SH, Weissman IL: The role of apoptosis in the regulation of hematopoietic stem cells: overexpression of BCL-2 increases both their number and repopulation potential. *J Exp Med* 191:253-263, 2000.

This study assessed the influence of apoptosis in regulating numbers of hematopoietic stem cells (HSCs). The study used a transgenic mouse strain, H2K-BCL-2, characterized by overexpression of BCL-2 in HSCs and other hematopoietic cells. Previous studies have shown that H2K-BCL-2 cells are protected from many different types of apoptosis-inducing challenges. Levels and cell cycles of HSC were investigated, along with plating efficiency, engraftment, and competitive reconstitution.

The HSCs of H2K-BCL-2-transgenic mice were morphologically similar to those of wild-type mice. However, HSC numbers in bone marrow were significantly greater—2.4-fold greater than in wild-type mice. In addition, significantly fewer HSCs from H2K-BCL-2 mice were in the S/G₂M phases of the cell cycle. The H2K-BCL-2 HSCs showed increased plating efficiency in vitro, and they engrafted at least as well as wild-type HSCs in vivo. The H2K-BCL-2 HSCs had a competitive advantage after reconstitution compared with wild-type HSCs. However, overexpression of BCL-2 did not translate into an increase in the life span of clones derived from “short-term” HSCs, which can self-renew or differentiate for only limited periods.

Findings in this transgenic mouse model show that overexpression of BCL-2, which protects against apoptosis, increases HSC numbers and repopulation potential.



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Thus, apoptosis appears to play a key role in limiting the numbers of blood stem cells. Further study is needed to identify the signals controlling the fate of HSCs.

Kawai T, Poncelet A, Sachs DH, et al: Long-term outcome and alloantibody production in a non-myeloablative regimen for induction of renal allograft tolerance. *Transplantation* 68:1767-1775, 1999.

A nonmyeloablative regimen was evaluated for use in inducing renal allograft tolerance in monkeys, including the effects of splenectomy and delayed renal transplantation. Cynomolgus monkeys underwent a basic preparative regimen, consisting of nonlethal total body irradiation, antithymocyte globulin, donor bone marrow transplantation, and 4 weeks of treatment with cyclosporine. No further immunosuppressive agents were given thereafter. One group of monkeys underwent kidney transplantation and splenectomy on the same day as marrow transplantation, day 0; another group underwent kidney transplantation but not splenectomy on day 0; a third group underwent splenectomy on day 0, followed 3 to 16 weeks later by kidney transplantation; the fourth group underwent both kidney transplantation and splenectomy at day 120. The animals were followed up for the development of anti-donor alloantibody and chimerism.

Chimerism developed in 11 of 13 monkeys undergoing kidney transplantation and splenectomy on day 0. Nine of these animals were long-term survivors, without signs of chronic vascular rejection. Just 1 of the long-term survivors showed alloantibodies. In contrast, all 3 monkeys undergoing immediate kidney transplantation without splenectomy developed alloantibodies and rejected the organ. Three of seven monkeys undergoing immediate splenectomy but delayed kidney transplantation developed chimerism; 2 of these had long-term survival without alloantibody production. When both splenectomy and kidney transplantation

were delayed, the organs were rejected soon after transplantation.

In this animal model, a nonmyeloablative regimen including donor bone marrow transplantation produces chimerism in most cases, and most of the chimeric animals do not develop antidonor antibodies. Deleting splenectomy from the regimen will reduce the rate of B-cell tolerance, leading to late antibody production and organ rejection. Bone marrow transplantation can induce donor-specific hyporesponsiveness without immediate transplantation of the donor kidney.

Kikuta T, Shimazaki C, Ashihara E, et al: Mobilization of hematopoietic primitive and committed progenitor cells into blood in mice by anti-vascular adhesion molecule-1 antibody alone or in combination with granulocyte colony-stimulating factor. *Exp Hematol* 28:311-317, 2000.

In a mouse model, antibody against vascular cell adhesion molecule (VCAM)-1 was studied for its ability to mobilize hematopoietic progenitor cells in blood. C57BL/6J mice received IV injections of anti-VCAM-1 and anti-very late antigen (VLA)-4 antibodies, 5 mg/kg, for 2 days. The effects on the numbers of colony-forming cells (CFCs) and colony-forming unit spleen (CFU-S) in blood were assessed. In a further experiment, recombinant human granulocyte colony-stimulating factor (G-CSF), 125 µg/kg twice daily, was given along with anti-VCAM-1 antibody.

Treatment with anti-VCAM-1 increased CFC numbers in blood more than 11-fold, although anti-VLA-4 had no effect. In addition, anti-VCAM-1 increased the number of CFU-S in blood by more than 21 times. Treatment with anti-VCAM-1 was associated with significantly reduced numbers of CFCs and CFU-S in the bone marrow and spleen. In animals treated with G-CSF plus anti-VCAM-1, CFC numbers increased by more than 141 times and CFU-S by 439 times.

Pretreatment with anti-VCAM-1 antibody is a promising approach to increase mobilization of hematopoietic progenitor cells into the blood. Even greater increases can be achieved through a synergistic effect of G-CSF plus anti-VCAM-1. Further study is needed to clarify the mechanisms by which hematopoietic stem cells and progenitor cells are mobilized.

Lowenthal RM, Tuck D, Tegg E, et al: Hemopoietic stem-cell harvesting and transplantation using G-CSF-primed BM: comparison with unprimed BM and G-CSF-primed PBSC. *Cytotherapy* 1:409-416, 1999.

Bone marrow primed with granulocyte colony-stimulating factor (G-CSF) was investigated as an alternative source of hematopoietic stem cells for transplantation. The retrospective analysis included 44 patients who underwent bone-marrow harvesting 6 days after priming with G-CSF, 5 µg/kg/d. These were compared with 44 patients who underwent standard bone marrow harvesting without priming. Most patients in both groups had a diagnosis of non-Hodgkin's lymphoma. Hematopoietic reconstitution outcomes were compared for 18 patients receiving G-CSF-primed bone marrow, 16 patients receiving unprimed bone marrow, and 14 patients receiving autologous peripheral blood stem cells (PBSCs) after priming with G-CSF and/or cyclophosphamide.

Bone marrow aspiration was considerably easier after G-CSF priming, reducing harvest time by about one-half hour. Priming with G-CSF also produced a larger number of cells, resulting in a larger number of cells available for transplantation. Time to achieve an unsupported platelet count of $20 \times 10^9/L$ or greater was 14 days with G-CSF-primed bone marrow, compared to 22 days with unprimed marrow and 10 days with primed PBSCs. The effects of G-CSF-primed marrow were also intermediate in terms of number of days that platelet transfusions were required and number of hospital days.

This experience demonstrates the value of G-CSF-primed bone marrow as a source of hematopoietic stem cells. Hematopoietic reconstitution with G-CSF-primed marrow is faster than with unprimed marrow, although not as fast as with primed PBSCs. This difference is probably related to the number of cells collected and the transplant-cell dose. Given its advantages in terms of collection and storage, G-CSF-primed bone marrow provides a useful alternative to PBSCs under certain clinical conditions.

Ludewig B, Ochsenbein AF, Odermatt B, et al: Immunotherapy with dendritic cells directed against tumor antigens shared with normal host cells results in severe autoimmune disease. *J Exp Med* 191:795-803, 2000.

A study was performed in mice to assess the potential for autoimmune effects occurring after vaccination with dendritic cells (DCs) targeted with immunogenic tumor antigens. Transgenic RIP-GP mice injected with DCs expressing a tumor antigen that was also present in pancreatic islet cells showed tumor control. However, they also developed autoimmune diabetes, which appeared and caused death rapidly. In this experiment, autoimmune diabetes developed under any circumstance in which CTL activity was prolonged.

In a second model, SM-LacZ mice were injected with DCs presenting a tumor antigen that was also present in arterial smooth muscle cells and car-

diomyocytes. This led to the development of severe arteritis, myocarditis, and—with repetitive treatment—dilated cardiomyopathy.

The findings raise an important problem in the use of antigen-expressing DCs for tumor treatment. Although immunization may destroy the tumor, it may also induce autoimmunity to a set of previously immunologically ignored antigens. When antigens that are not strictly tumor specific are used for antitumor vaccination with DCs, the window in which activated CTLs will reject the tumor without causing autoimmune disease appears to be a very small one. Thus autoimmune reactions may be a limiting factor in the use of DC immunotherapy.

McGlave PB, Shu XO, Wen W, et al: Unrelated donor marrow transplantation for chronic myelogenous leukemia: 9 years' experience of the National Marrow Donor Program. *Blood* 95:2219-2225, 2000.

The results of unrelated-donor bone marrow transplantation (BMT) in 1,423 patients with chronic myelogenous leukemia (CML) are presented. The transplantations were performed from 1988 to 1996 at 85 centers affiliated with the National Marrow Donor Program. Survivors were followed up for a median of 4 years. Eighty-one percent of donor-recipient pairs were matched for the HLA-A, -B, and -DR loci. Pretransplantation conditioning generally included total body irradiation plus cyclophos-

phamide, sometimes with other chemotherapy drugs.

The mean waiting time to find a donor decreased from 6.9 to 5.5 months during the period studied. Eighty-eight percent of recipients showed prompt engraftment, with a median engraftment time of 20 days. Engraftment failed in 10% of patients, and another 7% of evaluable patients developed late graft failure. One third of the patients developed grade III/IV acute GVHD. The 2-year rate of extensive chronic GVHD was 60%. The hematologic relapse rate was 9% overall, but only 6% in patients undergoing transplantation during the chronic phase. For the same group, The 3-year disease-free survival rate was 43%.

Other factors related to better disease-free survival included transplantation less than 1 year after diagnosis of CML, younger recipient age, recipient cytomegalovirus-seronegative status, and absence of grade III/IV acute GVHD. Among 157 patients with all favorable characteristics—age less than 35 years old, received transplant in chronic phase, and within 1 year of diagnosis, and recipients of HLA-matched marrow—3-year disease-free survival rate reached 63%.

The experience demonstrates the feasibility of unrelated donor BMT for patients with CML. The results of this procedure have improved with faster identification of an appropriate donor, along with measures to reduce the incidence and severity of complications. Young patients who receive transplants early and in the chronic phase achieve the best outcomes.

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