Blood and Marrow TRANSPLANTATION

REVIEWS

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Myelodysplastic Syndrome: The Transplanter's Quandaries

John R. Wingard, MD, Editor

Although the myelodysplastic syndromes (MDS) are not curable without hematopoietic cell transplant (HCT), advances in non-transplant therapies today offer considerable benefit to our patients. Over the years, prognostic algorithms have been developed and validated and these are useful guides to allow us to more accurately predict the likely trajectory of disease progression in a group of syndromes that have a notorious heterogeneity.

For lower risk MDS, advances in supportive care include optimization of when and how to administer hematopoietic growth factors and the growing recognition of the importance of iron (enough but not too much) and ways to deal with it. The introduction of demethylating agents has provided substantial benefit as well, controlling disease manifestations and delaying progression to leukemia and extending survival in some patients. Even older drugs such as anti-thymocyte globulin and newer drugs such as lenalidomide have their roles for certain subsets of patients. Other classes of new drugs are in the pipeline. Having more choices is indeed gratifying for a group of patients in whom just a few short years ago there were few good choices. With new choices come new dilemmas: for whom what choice is best for a given patient.

The quandaries of MDS for the transplant clinician are several: who should be offered HCT, when should transplant be done in those who need HCT, and how best to do the transplant. These are the topics addressed in this issue. A symposium presented at the 2008 BMT Tandem Meetings in San Diego, CA addressed a number of these thorny issues. While the answers are not yet in, there is a ferment of clinical research underway to provide guidance that we can apply to individual patients. Needed clinical trials are underway to developing safer and more effective HCT strategies. Hopefully more are to come.

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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.

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

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

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ASBMT News

Heslop Installed as President; Barrett Elected Vice President

Helen Heslop, MD, has been installed as president of the American Society for Blood and Marrow Transplantation. She is professor of medicine and of pediatrics and director of Adult Stem Cell Transplantation at the Center for Cell and Gene Therapy, Baylor College of Medicine, The Methodist Hospital and Texas Children's Hospital, Houston.

A. John Barrett, MD, section chief for Stem Cell Allotransplantation in the Hematology Branch of the National Heart, Lung and Blood Institute, Bethesda, is the newly elected and installed vice president, to become president in 2010.

Installed as secretary was Edward D. Ball, MD, professor of medicine and director and chief of the Blood and Marrow Transplantation Division and the Moores Cancer Center at the University of California San Diego, in LaJolla.

The installation of new officers and directors occurred at the society's annual meeting, the BMT Tandem Meetings, on Feb. 14 in San Diego. The election was by mail ballot among members of the society in December and January.

Newly elected and installed directors are:

- Kenneth R. Cooke, MD, of Case Western Reserve University in Cleveland
- H. Joachim Deeg, MD, of the Fred Hutchinson Cancer Research Center and the University of Washington in Seattle
- Steven M. Devine, MD, of the Ohio State University Comprehensive Cancer Center in Columbus

Claudio Anasetti, MD, was elevated to president-elect and will assume the presidency in 2009. He is professor of oncology and medicine at the University of South Florida, and program leader of the Blood and Marrow Transplant Program at the Moffitt Cancer Center and Research Institute, Tampa.

The new ASBMT president, Dr. Heslop, earned her medical degree with distinction at the University of Otago, completing training in medicine and hematology in New Zealand and the Royal Free Hospital in London. She was on faculty at St. Jude Children's Research Hospital before moving to Baylor in 1997.

She has broad administrative, clinical and research expertise. She is vice president of the Foundation for the Accreditation of Cellular Therapy (FACT) and a former member of the NIH Recombinant DNA Advisory Committee.

Dr. Heslop is an associate editor of Biology of Blood and Marrow Transplantation, co-editor of Bone Marrow Transplantation and an editorial board member of Blood. She was scientific program co-chair for the 2007 BMT Tandem Meetings in Keystone.

Her research focuses on immunotherapy of hematologic malignancies and reconstituting anti-viral immunity post transplant. She holds a Doris Duke Distinguished Clinical Scientist Award and is a member of the Association of American Physicians.

Membership Grows 5% to Record 1,508

ASBMT membership climbed 5% during 2007.

Increases occurred in all categories: member, associate member, affiliate member and in-training member.

Health professionals outside the United States and Canada comprise 13% of ASBMT members.

Attendance at BMT Tandem Meetings in San Diego Exceeds 2,500

Registration for the BMT Tandem Meetings in San Diego was 2,501 – 34% greater than the previous year in Keystone and 23% above the record set in 2006 in Honolulu. Attendees came from 47 countries.

Six Abstracts Chosen as Best of BMT Tandem Meetings

A total 509 abstracts from 31 countries were accepted for the 2008 BMT Tandem Meetings.

Six of the abstracts were selected for awards by the abstract review committees.

Recipients of the ASBMT Best Abstract Awards for Basic Science Research were:

- Hisham Abdel-Azim, MD, Childrens Hospital Los Angeles – *Targeted in vivo Expansion of Human Multipotent and Lymphoid Progenitors*
- Yishay Ofran, MD, Dana-Farber Cancer Institute, Harvard Medical School, Boston – *Identification of Human Minor Histocompatibilty Antigens by Combining Bioinformatic Prediction of Peptide Epitopes with Validation of T Cell Reactivity in Patient Blood Samples after Allogeneic Hematopoietic Stem Cell Transplantation*
- Pablo Ramirez, MD, Washington University, St. Louis – *Mobilization of Normal Mouse Progenitors and Acute Promyelocytic Leukemia Cells with Inhibitors of CXCR4 and VLA-4 in Splenectomized and Unsplenectomized Mice*

Each received a $1,000 prize.

Recipients of the CIBMTR Best Abstract Awards for Clinical Research were:

- Herrad Baurmann, MD, Deutsche Klinik fuer Diagnostik, Wiesbaden, Germany – *Risk Factors for Allogeneic Stem Cell Transplantation in Patients with Myelofibrosis with Myeloid Metaplasia*
- Gregory A. Hale, MD, St. Jude Children's Research Hospital, Memphis – *Long-Term Follow-Up of Administration of Donor-Derived EBV-Specific CTLs to Prevent and Treat EBV Lymphoma after Hemopoietic Stem Cell Transplant*
- Nabil Kabbara, MD, Eurocord, Paris, France – *A Multicentric Comparative Analysis of Outcomes of HLA Identical Related Cord Blood and Bone Marrow Transplantation in Patients with Beta-Thalassemia or Sickle Cell Disease*

Each also received a $1,000 prize. The clinical research awards are supported by a grant from Gambro BCT.

Recordings Available for San Diego Presentations

Audio CDs, synchronized audio/visual CDs and MP3 downloads can be purchased for BMT Tandem Meetings plenary and concurrent scientific sessions, symposia and oral abstract sessions.

Also available are the recordings of many presentations at the parallel conferences of the transplant nurses, BMT pharmacists, BMT center administrators and clinical research professionals.

To location and purchase programs, visit ***www.asbmt.org/cibmtr/Tandem***.

BMT Tandem Meetings Abstracts Are Searchable Online

Abstracts accepted for the BMT Tandem Meetings were published in the February 2008 issue of *Biology of Blood and Marrow Transplantation* (Vol. 14, No. 2, Supplement).

They also are indexed and accessible online on ***www.abstracts2view.com/tandem.***

Maximizing Treatment Outcomes for MDS in the Transplant Patient

Adapted from a continuing medical education symposium presented at the 2008 BMT Tandem Meetings on February 13, 2008, in San Diego, California.
This program is supported by an educational grant from Celgene.

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Overview

This publication will review expert presentations of clinical data in conjunction with patient case reports that establish the role of transplantation and drug therapy in treating patients with myelodysplastic syndromes (MDS). It covers data supporting various treatment strategies for MDS, including pharmacologic manipulation, with the goal of defining ways to maximize treatment outcomes in the transplant patient.

Target Audience

This activity is intended for transplantation physicians and allied health professionals.

Learning Objectives

- Describe the current treatment options for MDS in the transplant patient
- Summarize the optimal regimen pretransplant to achieve disease control
- Debate when to transplant the MDS patient: early versus late
- Discuss the outcomes of allogeneic transplantation for refractory MDS and AML
- Formulate treatment options based on the possible role of hypomethylating agents as maintenance therapy after allogeneic transplantation

Accreditation Statement

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

Designation of Credit

The Medical College of Wisconsin designates this educational activity for a maximum of 1.0 *AMA PRA Category 1 Credits*™. Physicians should only claim credit commensurate with the extent of their participation in the activity.

Disclaimer

This material has been prepared based on a review of multiple sources of information, but it is not exhaustive of the subject matter. Participants are advised to critically appraise the information presented, and are encouraged to consult the abovementioned resources as well as available literature on any product or device mentioned in this program.

Disclosure of Unlabeled Uses

This educational activity may contain discussion of published and/or investigational uses of agents that are not approved by the US Food and Drug Administration. For additional information about approved uses, including approved indications, contraindications, and warnings, please refer to the prescribing information for each product, or consult the Physician's Desk Reference.

Faculty Disclosure

Consistent with the current Accreditation Council for Continuing Medical Education policy, the CME Provider must be able to show that everyone who is in a position to control the content of an individual educational activity has disclosed all relevant financial relationships. The CME Provider has a mechanism in place to identify and resolve any conflicts of interest discovered in the disclosure process. The presenting faculty members have all made the proper disclosures, and the following relationships are relevant:

Marcos J. de Lima, MD: has received grant and research support from Celgene.

Madan H. Jagasia, MBBS: has received honoraria from and is a speaker for Celgene.

Bart L. Scott MD: has received honoraria from and is a speaker for Celgene and MGI Pharma.

Maximizing Treatment Outcomes for MDS in the Transplantation Patient

Bart L. Scott, MD

Major issues currently being investigated in stem cell transplantation for myelodysplastic syndrome (MDS) include timing of transplantation, type of transplant, and whether or not pretransplantation chemotherapy is beneficial.

Timing of Transplantation

In regard to the timing of transplantation, Cutler et al [1] reported their analysis of outcomes in 3 different groups of patients: 184 who did not receive transplants, 260 patients who underwent transplantation for MDS, and 230 patients who underwent transplantation after progression to acute myeloid leukemia (AML) from preceeding MDS. Disease stage was determined by use of the International Prognostic Scoring System. In patients who had low- or intermediate-1–risk disease, a delay in stem cell transplantation led to a gain in life expectancy, whereas in patients with high- or intermediate-2 risk disease, delay of stem cell transplantation led to a loss in life expectancy (Figure 1).

Some specific issues may have affected this analysis. All of the transplantation patients received bone marrow stem cell grafts, all of the donors were related, and all of the conditioning was myeloablative. Because all of the conditioning was full-dose intensity, the results do not necessarily apply to nonmyeloablative conditioning. Although patients with Low or Int-1 risk disease may benefit from a delay in transplantation, exactly when they should be considered for stem cell transplantaion remains unclear. Presumably, transplantation should be considered in patients with low or intermediate-1 risk at the time of a significant clinical event such as progressive cytopenias, an increase in bone marrow myeloblast percentage, transfusion dependence, or the emergence of new cytogenetic abnormalities. The reasoning behind the strategy of delaying transplantation is that mortality associated with the stem cell transplantation procedure itself leads to worse outcomes in patients with low- or intermediate-1–risk disease.

Conditioning Regimens

Conditioning regimens used in transplantation for MDS encompass a broad spectrum, with a wide range of myeloablative versus nonmyeloablative properties. Treating MDS patients involves ongoing efforts to better characterize patients to determine what type of conditioning regimen will be most effective for each individual patient.

The risk of mortality associated with stem cell transplantation has led to the use of reduced-intensity conditioning regimens, but the decreased nonrelapse mortality is associated with increased relapse rates. In a multicenter retrospective study conducted by the EBMT, Martino et al [2] analyzed outcomes according to 2 types of conditioning regimens, reduced-intensity and standard myeloablative (or high-dose) conditioning, in 836 patients with MDS who underwent transplantation with an HLA-identical sibling donor. Multivariate analysis results indicated that in the group who received reduced-intensity conditioning (n = 215) the 3-year relapse rate was significantly increased but the 3-year nonrelapse mortality rate was decreased. These patients were older and had more adverse pretransplantation variables than the patients who received standard myeloablative conditioning (n = 621). Because of the higher risk of relapse associated with reduced-intensity conditioning, the investigators conclude that reduced-intensity conditioning should not routinely be considered for patients who are candidates for myeloablative conditioning outside of a clinical trial. The major issue with this analysis was the retrospective nature of the analysis and the subsequent lack of ability to control for factors that would inherently bias the results, such as the advanced age or co-existing comorbidities present in the patients who underwent reduced-intensity conditioning. Additionally, there were a variety of regimens included in both the reduced-intensity conditioning and standard myeloablative conditioning.

Another area of investigation is reducing the toxicity of standard myeloablative regimens. Bornhauser et al investigated the use of a modified version of the standard myeloablative regimen of targeted busulphan and cyclophosphamide in which cyclophosphamide was replaced with fludarabine to decrease toxicity and facilitate donor engraftment [3]. They conducted a clinical trial using a regimen of intravenous fludarabine and oral busulfan before transplantation of allogeneic hematopoietic stem cells in 48 patients with chronic myelogenous leukemia and 38 patients with MDS. Engraftment was achieved in all patients, and the day-100 regimen-related mortality was 7%. With a median follow-up of 18 months (range, 13-27 months), the probabilities of overall survival, disease-free survival, and nonrelapse mortality were 42.4%, 34.9%, and 24%, respectively. These data indicate that the combination of fludarabine and targeted busulfan is sufficiently immunosuppressive to facilitate engraftment of blood stem cells from HLA-matched siblings and unrelated donors and that further studies of fludarabine and targeted busulfan are warranted in standard-risk patients. The use of a reduced-toxicity regimen of intravenous fludarabine and busulphan was also investigated at the M.D. Anderson Cancer Center, where results suggested this regimen was effective in patients with acute myelogenous leukemia (AML) or MDS [4]. Remarkably

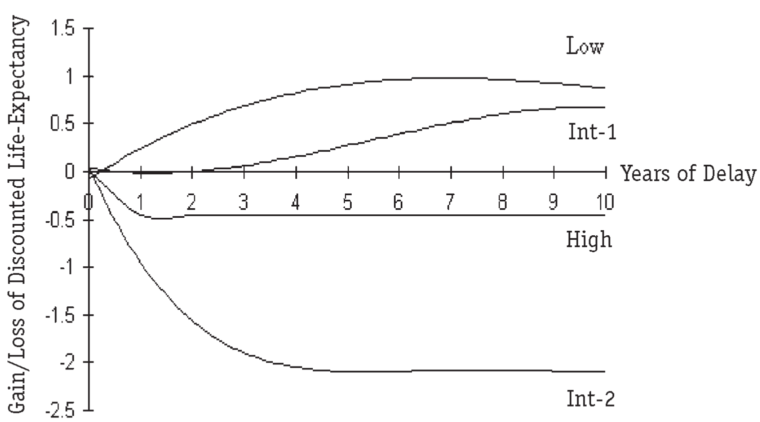

Figure 1. Timing of transplantation and life expectancy in relation to IPSS disease risk score. Int indicates intermediate. This research was originally published in Blood. Cutler CS. A decision analysis of allogenic bone transplantation for the myelodysplastic syndromes: delayed transplantation for low-risk myelodysplasia is associated with improved outcome. *Blood*. 2004;104:579-585. © the American Society of Hematology.

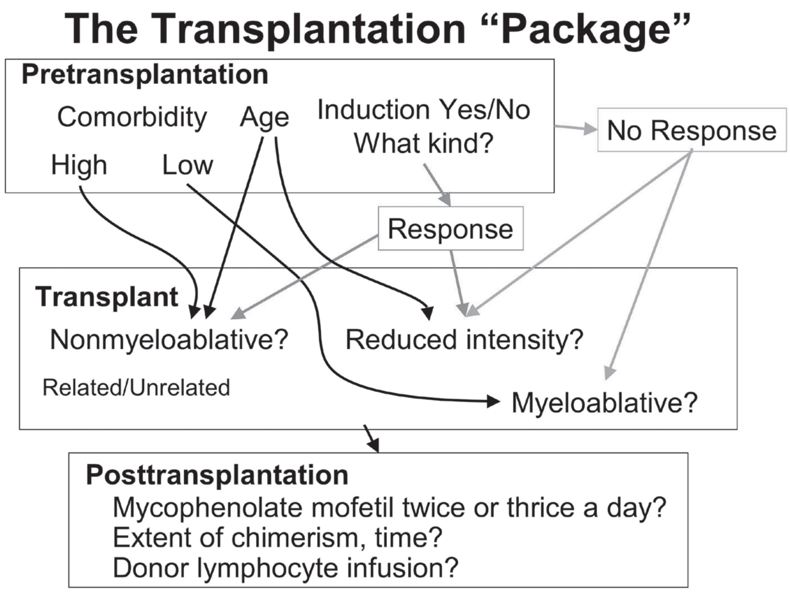

Figure 2. Factors involved in treatment planning for MDS patients undergoing allogeneic stem cell transplantation.

the nonrelapse mortality at 1-year was only 3% for this regimen with an overall survival of 65% and diseae-free survival of 52%.

A retrospective analysis comparing the results of nonmyeloablative to myeloablative stem cell transplantation at the Fred Hutchinson Cancer Research Center showed no difference in posttransplantation outcomes associated with the conditioning regimen in those patients who underwent transplantation while their disease was in remission, indicating the possibly that conditioning intensity, in and of itself, is not an important factor in patients who are in remission at the time of transplantation [5]. Like the EBMT analysis, this study was subject to the inherenet bias in a retrospective analysis. Additionally, there were a smaller number of patients included in this analsyis and therefore, the equivalence between non-myeloablative and myeloablative conditioning may have occurred as a result of type II error (the lack of power to detect a difference). In a study conducted by Alyea et al [6], patients undergoing nonmyeloablative transplantation were at high risk for treatment-related complications because they were older and more likely to have received previous or myeloablative transplantation. The results of this study demonstrated that although dose intensity played a significant role disease control after transplantation, this benefit was negated by increasing treatment-related mortality and suggested that nonmyeloablative transplantation is a reasonable alternative for patients with advanced AML and MDS at high risk for complications after myeloablative transplantation.

Pretransplantation Chemotherapy

Investigation of chemotherapy before transplantation for MDS treatment includes the use of conventional cyclophosphamide-based regimens and newer agents, particularly the methyltransferase inhibitors.

A retrospective analysis performed at the Fred Hutchinson Cancer Research Center investigated pretransplantation induction chemotherapy with cyclophosphamide and posttransplantation relapse in patients with advanced MDS and AML. This study showed no evidence of a benefit in posttransplantation outcome associated with prior induction chemotherapy [7]. A prospective analysis of posttransplantation outcomes in patients randomized to either receive or not receive induction chemotherapy has yet to be performed.

Among the newer agents that have proven useful in MDS treatment are the cytosine analogs, decitabine and azacitidine, which work by insertion into DNA and absorbtion of DNA methyl transferase, leading to global hypomethylation. A landmark study of azacitidine by the Cancer and Leukemia Group B [8,9] showed significant differences in complete remissions, partial remissions, and "improved" categories in the azacitidine-treated group compared with the group receiving only supportive care, with overall response rates of 60% versus 5%. There was a marked reduction in the frequency of AML transformation in patients treated with azacitidine. A number of different quality-of-life parameters, including physical functioning, fatigue, dyspnea, and overall quality of life, also showed improvement in the azacitidine-treated group [10]. Further analysis of transfusion data showed that of the responding patients, 86% of those treated initially with azacitidine and, after crossover, 93% of those treated become red cell transfusion independent.

A recent study showed a statistically significant increase in overall survival in MDS patients who received azacitidine [11]. A promising new area for further investigation is the incorporation of these new agents into the transplantation regimen.

Conclusions

Questions to be addressed in stem cell transplantation treatment for MDS patients are timing of transplantation, choice of a preparative regimen, and the role of pretransplantation chemotherapy (Figure 2) Optimal timing of transplantation remains a controversial area. Disease stage is known to be an important factor in the choice of preparative regimen, but randomized clinical trials are needed to evaluate dose intensity. As for the role of pretransplantation chemotherapy, retrospective analysis has not shown induction chemotherapy to be beneficial, but newer agents such as DNA methyltransferase inhibitors may play a role.

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Disease Control Prior to Transplantation: Does It Matter?

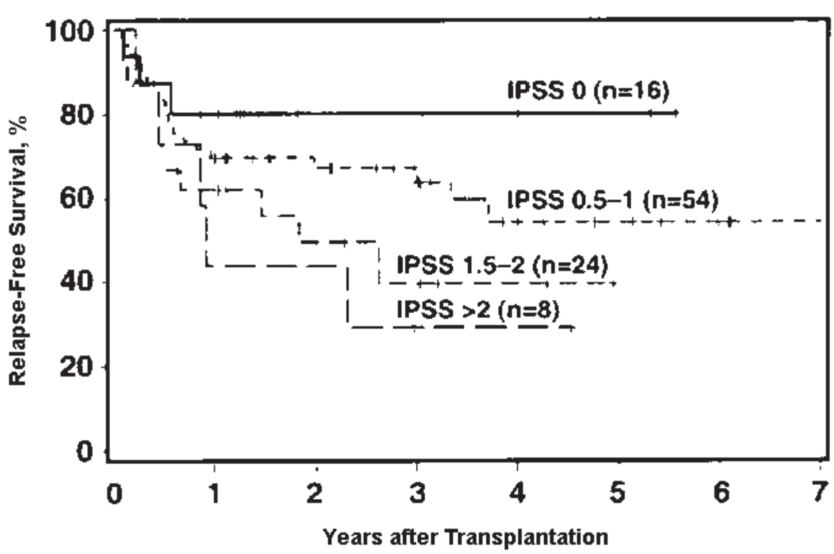
Madan H. Jagasia, MBBS, MS

Most transplantation clinical studies start the clock when the patient starts the transplantation procedure. Treatment options during the pretransplantation period must also be investigated, however, to find ways to optimize disease control prior to transplantation, thus maximizing the benefits of transplantation.

The goals of pretransplantation therapy are controlling disease, preventing the worsening of comorbidity, and minimizing infection. Once transplantation is chosen as a treatment option, decisions must be made regarding the timing of transplantation and selection of the optimal induction regimen. The choice of induction regimen involves favorable modulation of the balance between graft-versus-host disease (GVHD) and the graft-versus-tumor (GVT) effect.

Disease Status and Regimen Intensity

Assessment of disease status at diagnosis is the first step in planning treatment. The International Prognostic Scoring System (IPSS) has been validated for use in risk stratification in MDS patients, and IPSS at diagnosis remains an independent prognostic factor for predicting the outcome after an allogeneic transplantation [1] (Figure).



Kaplan-Meier plot showing the outcome of 109 patients who underwent transplantation with related or unrelated donors using an ablative regimen with targeted oral busulfan and cyclophosphamide. Pretransplantation marrow blast score and IPSS were the most important predictors of survival. The day 100 and 3-year nonrelapse mortality rates were 16% and 31%, respectively, resulting in a 3-year relapse-free survival of 56% with related-donor and 59% with unrelated-donor transplantation. Adapted from [1]. Courtesy of J. Deeg.

The effect of conditioning regimen intensity on transplantation outcomes is an area of ongoing investigation. Martino and colleagues investigated treatment outcomes in 836 MDS patients who received an HLA-identical, matched-related transplant. Before transplantation, 621 of these patients underwent a standard ablative preparation regimen and 215 underwent a reduced-intensity regimen. Even though all of the patients in the reduced-intensity group were older than those in the ablative group, the reduced-intensity group had a significantly lower nonrelapse mortality rate compared to the ablative group. This result was offset, however, by higher relapse rates in the reduced-intensity group, thus leading to similar rates in both groups for 3-year overall survival and progression-free survival [2].

Multivariate analysis results for this study revealed that patient age of more than 50 years was an independent prognostic indicator for nononrelapse mortality. Similarly, patients who had not received prior treatment, or who had been treated but were not in first complete remission, had higher nonrelapse mortality.

Comorbidity is another important variable that affects nonrelapse transplantation mortality. The Hematopoietic Cell Transplant Comorbidity Index (HCT-CI), recently developed by Sorror et al [3], is a refinement of the earlier Charleson Comorbidity Index [4]. The HCT-CI (Table 1) is a tool for risk stratification for nonrelapse mortality. Diaconescu and colleagues investigated HCT-CI along with disease risk status in a large cohort of patients, and showed similar outcomes after myeloablative and nonmyeloablative transplantation [5]. Using the HCT-CI to control for comorbidity status, they also found that the outcome after nonmyeloablative transplantation is similar in recipients of related- and unrelated-donor transplants.

Based on these data, it is reasonable to make a statement that when controlled for comorbidity score and disease risk, transplantation outcome may be similar irrespective of regimen intensity and donor status.

Table 1. Comorbidities Included in the HCT-CI Scores*

Comorbidity	Definitions of Comorbidities	HCT-CI Weighted Scores
Arrhythmia	Atrial fibrillation or flutter, sick sinus syndrome, or ventricular arrhythmias	1
Cardiac	Coronary artery disease, congestive heart failure, myocardial infarction, or ejection fraction ≤50%	1
Inflammatory bowel disease	Crohn disease or ulcerative colitis	1
Diabetes	Requiring treatment with insulin or oral hypoglycemics but not diet alone	1
Cerebrovascular disease	Transient ischemic attack or cerebrovascular accident	1
Psychiatric disturbance	Depression or anxiety requiring psychiatric consult or treatment	1
Hepatic, mild chronic hepatitis	Bilirubin > ULN to 1.5 × ULN, or AST/ALT > ULN to 2.5 × ULN	1
Obesity	Patients with a body mass index > 35 kg/m2	1
Infection	Requiring continuation of antimicrobial treatment after day 0	1
Rheumatologic	SLE, RA, polymyositis, mixed CTD, or polymyalgia rheumatica	2
Peptic ulcer	Requiring treatment	2
Moderate/severe renal	Serum creatinine > 2 mg/dL, on dialysis, or prior renal transplantation	2
Moderate pulmonary	DLco and/or FEV1 66%-80% or dyspnea on slight activity	2
Prior solid tumor	Treated at any time point in the patient's past history, excluding nonmelanoma skin cancer	3
Heart valve disease	Except mitral valve prolapse	3
Severe pulmonary	DLco and/or FEV1 ≤65% or dyspnea at rest or requiring oxygen	3
Moderate/severe hepatic	Liver cirrhosis, bilirubin > 1.5 × ULN, or AST/ALT > 2.5 × ULN	3

*Adapted from [3]. ULN indicates upper limit of normal; ALT/AST, aspartate aminotransferase/alanine aminotranferease, SLE, systemic lupus erythmatosis; RA, rheumatoid arthritis; CTD, connective tissue disease; DLco, diffusion capacity of carbon monoxide; FEV, forced expiratory volume.

Transplantation Timing

Controversy still exists as to whether all eligible patients should undergo transplantation at diagnosis or whether a select group may be better served by observation and nontransplantation treatment strategies for a variable period of time. Cutler et al have reported that for patients with low- and intermediate-1–risk disease, as assessed by use of the IPSS, life expectancy is better when transplantation is delayed. On the other hand, patients with IPSS intermediate-2 and high-risk MDS have the best results with earlier transplantation [6].

MDS is uncommon in young patients, but when it occurs these individuals are often offered transplantation early in their disease course, because transplantation is considered a definitive therapy. A recent study by Kuendgen and colleagues [7] compared the outcome of 232 patients younger than 50 years to approximately 2500 patients older than 50 years. Even in patients younger than 50 years, IPSS low-risk disease stage predicted for a survival rate of 86% at 20 years. The median survival rate in the low-risk group has not been reached. For IPSS intermediate-1 disease, median survival was 176 months, and for intermediate-2 and high-risk disease, 8 and 7 months, respectively. Patients who received AML-type chemotherapy or received an allogeneic transplant were censored for this analysis. These results indicate that patients younger than 50 years who have low-risk or intermediate-risk disease have excellent survival, even without an allogeneic transplantation, and thus these patients should probably be offered a watch-and-wait policy or intervention with nontransplantation strategies until there is disease progression.

Although the IPSS scoring system (Table 2) has been used as a standard prognostic tool for predicting MDS survival and risk of transformation into AML, the system has limitations because it is not time dependent and does not incorporate the World Health Organization (WHO) prognostic histologic criteria, which expand on the earlier French-American-British classification system. Malcovati et al [8] have validated a WHO classification-based prognostic scoring system (WPSS) that incorporates the WHO pathologic classification, along with cytogenetics and transfusion requirements (Table 3). Their group has previously shown that increased transfusion requirement is an independent, negative prognostic indicator of survival in MDS patients [9]. MDS patients are now risk-stratified into 5 categories based on the WHO-IPSS score: very low (0), low (1), intermediate (2), high (3 or 4), and very high score (5 or 6).

Pretransplantation Treatment with Chemotherapy or Other Agents

Pretransplantation treatment options for MDS patients include conventional AML cytotoxic chemotherapy, demethylating agents, and other agents. AML chemotherapy for MDS is generally not effective because it is associated with a significant rate of toxic deaths and infections [10]. Decitabine, a demethylating agent, is approved by the US Food and Drug Administration (FDA) for treatment of MDS. Various dose regimens have been used, and recent data from M.D. Anderson Cancer Center suggest that the best response is obtained with a dosage of 20 mg/m^2 daily for 5 days, cycled every 4 weeks. No data have been reported on the use of this agent in a systematic manner prior to transplantation.

Clofarabine is a second-generation purine nucleoside analog that is active in AML as a single agent, or in combination. The use of clofarabine in MDS has been limited; however, there have been reports of its therapeutic activity in patients in whom treatment with demethylating agents has failed. Like decitabine, clofarabine has not been systematically studied in the pretransplantation setting [11].

Azacitidine is a demethylating agent approved by the FDA for the treatment of all subsets of MDS. Recent data in patients with intermediate-2 and high-risk MDS show a survival advantage with the use of azacitidine compared to best conventional care. Data from Fenaux et al [12] show that patients treated with azacitidine had statistically superior median survival compared to patients given conventional care in all risk groups that were studied. Patients in the azacitidine arm had a prolonged time to AML and death. Patients in the azacitidine group had a higher incidence of red blood cell transfusion independence and a 33% reduction in infections requiring intravenous antibiotics. The conventional-care group consisted of 3 cohorts: best supportive care, low-dose cytarabine, and conventional AML chemotherapy. Azacitidine was superior to best supportive care; however, the difference in overall survival did not reach statistical significance when azacitidine was compared to low-dose cytarabine and chemo-

Table 2. IPSS Score for MDS Staging

Prognostic Variable	0 Points	0.5 Points	1 Point	1.5 Points	2 Points
Bone marrow blasts, %	<5	5-10	–	11-20	21-30
Karyotype	Good	Intermediate	Poor	–	–
Cytopenias	0/1	2/3	–	–	

Table 3. WPSS*

Variable	0	1	2	3
WHO Category	RA, RARS, 5q-	RCMD, RCMD-RS	RAEB-1	RAEB-2
Karyotype	Good	Intermediate	Poor	–
Transfusion requirement	No	Regular	–	–

*RA indicates refractory anemia; RARS, RA with ringed sideroblasts; RCMD, refractory cytopenia with multilineage dysplasia; RAEB, RA with excess blasts.

therapy. This analysis was limited by a small sample size.

Although azacitidine treatment prior to transplantation has not been studied in a prospective manner, a retrospective series conducted at the Moffitt Cancer Center looked at 14 patients who received pretransplantation azacitidine and 20 patients who did not [13]. These patient groups were matched for age, IPSS subtype at diagnosis, and IPSS subtype just prior to transplantation. An interesting finding was noted. None of the 14 patients who received pretransplantation azacitidine relapsed, compared to 8 of 20 patients who did not receive pretransplantation azacitidine. The 1-year overall survival and progression-free survival did not reach statistical significance; however, so this finding needs to be reexamined and revalidated.

Can demethylating agents administered prior to transplantation favorably modulate the GVHD and GVT effect balance? Review of the recent literature suggests that epigenetic modification is an important regulatory mechanism for optimal performance of the immune system. Recent data suggest that DNA demethylation in the human FOXP3 locus differs in regulatory T cells compared to conventional FOXP3-positive activated T cells [14]. Regulatory T cells (CD4+CD25-high Fox p3+) have the ability to down-modulate GVHD but probably also preserve the GVT effect. Thus regulatory T cells are an important subset of cells felt to be immunosuppressive and to modulate GVHD. Multiple investigators have looked at regulatory T-cell numbers as a predictor of GVHD. Epigenetic modification of regulatory T cells represents another layer of complexity as to how the immune system is regulated after allogeneic stem cell transplantation. Similarly, dynamic changes in histone methylation may perform a regulatory function during the differentiation of T-helper type-2 cells and may do so by epigenetic modification of the loci for important cytokines such as interferon γ and interleukin (IL)-2, IL-4, and IL-12 [15]. Thus it is likely that the use of demethylating agents during the pretransplantation period controls not only the disease but modulates the donor-host immune interaction after allogeneic stem cell transplantation. Evidence clearly indicates that there is crosstalk between demethylation and the immune system, and this interaction must be studied in detail in the context of GVHD and the GVT effect.

As we move forward into clinical trials for new treatment agents, the endpoint cannot be just disease control. A composite endpoint must be used that allows us to look at maximizing the number of patients whose treatment is optimized throughout their disease course, beginning with appropriate timing of allogeneic transplantation, maintaining adequate disease control without worsening of comorbidity, and avoiding major infections prior to transplantation.

Conclusions

In the vast majority of MDS patients, pretransplantation optimization of treatment is essential to ensure successful outcomes. It is obvious that a series of clinical trials will be needed to determine whether pretransplantation therapy make a difference in MDS. In my view, such a clinical trial endpoint needs to be a composite consisting of the number of patients reaching transplantation with adequate disease control, with no worsening of their comorbidity score and with minimal infections prior to transplantation. Secondary endpoints would be outcome posttransplantation, including nonrelapse mortality, overall survival, disease-free survival, and GVHD incidence and severity. We hope that in the near future we can move in this direction and start looking at these questions in a systematic manner. Demethylating agents may allow us to adequately control the disease without a detrimental impact on the comorbidity score; however, this possibility needs further scientific investigation. The role of pretransplantation therapy in GVHD and the GVT effect also requires further scientific elucidation.

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Preventing Relapse and Enhancing the Graft-versus-Leukemia Effect through Pharmacologic Manipulation

Marcos J. de Lima, MD

Acute myelogenous leukemia (AML) and myeloysplastic syndrome (MDS) are diseases that are strongly associated with aging. Unfortunately, the older patients are, the less likely they are to enter remission, so ultimately most patients will not be in remission or will have very short complete remissions of their diseases. Given that disease refractoriness is a concern in the majority of patients with MDS or AML, it is not acceptable to assume that these patients are not candidates for transplantation.

Treatment of refractory disease with hypomethylating agents such as azacitidine may maximize the graft-versus-leukemia (GVL) effect, prolong remission, and treat minimal residual disease. Unfortunately, however, these agents may also increase the incidence and severity of graft-versus-host disease (GVHD) and compromise graft function and immune recovery, and they are associated with other side effects.

This report presents current result of an ongoing trial to determine dose and schedule of maintenance therapy with low-dose azacitidine after allogeneic hematopoietic stem cell transplantation in patients with high-risk AML or MDS.

Relapse in AML and MDS

The frequently documented association of aging with resistant disease and the presence of comorbid conditions precludes the use of fully ablative transplantation in most patients 60 years old or older. Disease relapse remains the major cause of treatment failure after allogeneic transplantation for relapsed AML or MDS. In patients whose disease is truly refractory, treatment results are very poor, with only 10%-20% long-term survival. Even for refractory patients, however, complete remission rates after allogeneic transplantation are as high as 90%, but these remissions are usually short lived, with most relapses occurring during the first 3-4 months, although some may occur as quickly as 90 days posttransplantation (Figure 1). Thus any intervention designed to prevent relapse must be implemented very early.

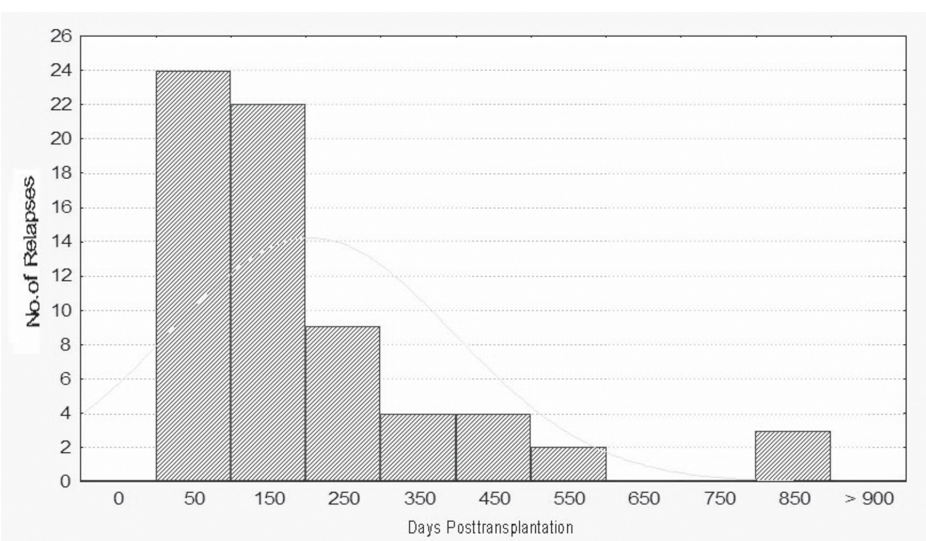

Figure 1. Occurrence of relapse after hematopoietic stem cell transplantation in patients with refractory AML or MDS. Most relapses occur in the early posttransplantation period.

Hypomethylating Agents in MDS Treatment

Hypermethylation in malignant cells is associated with silencing of regulatory genes. In particular, the P15 promoter region has been found to be hypermethylated in 65% of AML and 38% of MDS patients.

Hypomethylating agents exert an overall antimalignancy effect through inhibition of DNA methyltransferase. Very often a significant minority of treated patients will have a reversal of their malignant phenotype toward a more mature phenotype. This change occurs through an epigenetic mechanism that restores activity to tumor-suppressor genes and other genes involved in maturation that were otherwise silent. Phenotypic modifications of leukemic cells induced by hypomethylating agents, including reduction of CD13 and CD33 expression, increase antigenic density of surface determinants of mature myeloid cells such as CD16 and CD11c, and increase expression of major histocompatibility complex–class I molecules, HLA-DR, and β-2–microglobulin on the surface of cancer cells. These immunologic actions may increase the GVL effect and eliminate minimal residual disease [1-3]. Pioneering studies with the hypomethylating agent decitabine showed that administration of low doses of this agent, much less than needed for optimal myelosuppression, produced marked clinical benefit in patients with MDS, improving blood counts and delaying the time to disease progression [4].

Posttransplantation Hypomethylating Agents to Delay or Prevent Disease Recurrence

We postulated that the use of posttransplantation therapy with the hypomethylating agent azacitidine will decrease the relapse rate after allogeneic transplantation, giving time for posttransplantation GVL effects to occur. On the other hand, the same mechanisms might lead to adverse effects such as increased GVHD, compromised immune recovery, or direct toxicity, which may compromise graft function, particularly in the posttransplantation period. Thus dosing is an important issue, and we are also investigating the possibility that low doses may be as effective as higher doses and be better tolerated early after transplantation, when myelosuppression is a major risk.

Patients in this nonrandomized dose- and schedule-finding study initially received azacitidine at doses of 8, 16, and 24 mg/m^2. When these doses were found to be well tolerated, the trial was amended to include doses of 32, 40, 48, and 56 mg/m^2.

A preliminary study (n = 9) indicated a complete remission rate of 30% with doses of 16 or 24 mg/m^2 for 5 days, with minimal toxicity. The complete remission rate was 15%, and there were 30% responders. These preliminary results provide evidence of therapeutic activity for azacitidine given at a very low dose of 16 to 32 mg/m^2 for 5 days (the recognized dose approved by the US Food and Drug Administration for MDS treatment is 75 mg/m^2 for 7 days). The duration of treatment is undetermined at this point, but outside the clinical trial scenario, we have 5 patients at M.D. Anderson Cancer Center who have taken the drug at low doses without major side effects for more than a year post-transplantation. So growing evidence seems to support the use of low-dose azacitidine, but whether and the extent to which this treatment is effective remains to be proven.

The central hypothesis of our current trial is that azacitidine will decrease the relapse rate after allogeneic transplantation using a conditioning regimen of gemtuzumab ozogamicin, fludarabine, and melphalan, which is our backbone regimen for older patients who have suffered relapsed disease. Fludarabine, an important new drug used in transplantation preparative regimens, inhibits DNA repair and acts synergistically when given with an alkylating agent, inducing cytotoxicity and apoptosis. A subhypothesis of our investigation is that low doses of azacitidine may be as effective as higher doses and would be better tolerated early after transplantation.

Patients meeting study criteria are 12-75 years old (priority for patients older than 55-60 years or with comorbidities) with a diagnosis of AML not in first complete remission or MDS with an International Prognostic Scoring System (IPSS) score of intermediate-2 or high-risk, and who are ineligible for conventional high-dose chemotherapy.

The design of this study differs from that of most studies. A classic phase I study seeks to determine the optimum dose, but we are investigating an additional dimension by also trying to define a dosing schedule, ie, not just how much to give but how often it can be given. Initially the doses of azacitidine were 8, 16, and 24 mg/m^2, but the trial was amended last year to increase doses to 32, 40, and 56 mg/m^2 for 5 days. Essentially, we are monitoring time to toxicity for given doses delivered a given number of times; for example, 3 different doses, of 8, 16 and 24 mg/m^2, and 4 different schedules, ie, 4 opportunities for delivery of the drug (Figure 2).

One of our assumptions is that the alteration of methylation status by azicitidine can eradicate minimum residual disease. Determination of minimum residual disease is accomplished by measurement of the global DNA methylation status of the donor and the recipient using the LINE (long interspersed nucleotide elements) assay (bisulfite pyrosequencing) [5]. Gene-specific methylation changes will allow the analysis of epigenetic chimerism during the therapy. We will be analyzing the following genes: p15, MDR1, p57KIP2, THBS2, and p73, and our ultimate goal is to monitor immune recovery.

Patients are receiving gemtuzumab ozogamicin, 2 mg/m^2, on pretransplantation day 12, and then they go to receive the fludarabine and melphalan regimen. They receive tacrolimus and minimethotrexate for GVHD prophylaxis, and in accordance with M.D. Anderson policy, rabbit antithymocyte globulin is given to recipients of unrelated-donor or mismatched-related–donor transplants.

Patients receive up to 4 monthly cycles of azacitidine for 5 days, and we are currently transitioning the dose from 32 to 40 mg/m^2. So initially patients received 8 mg/m^2 for 1 cycle, and the dose has progressively increased. A list of requirements for receiving azacitidine is presented in the Table. Because azacitidine is being given as maintenance therapy, patients receiving azacitidine must be in remission after transplantation. They also must have good kidney and liver function, a functioning graft, and no bleeding. Patients meeting these criteria are assigned a specific dose and schedule of azacitidine, which is started in the sixth week after transplantation, on day 42 or so.

At the time of this report, 60 patients were included in the trial. The median age was 58 years; 90% of the study patients had active disease at the time of transplantation and 15% had received a previous allogeneic transplant. We have been able to administer azacitidine to approximately 60% of the patients; 17 patients (42%) were not eligible to receive azacitidine at day +42. Reasons for not receiving drug treatment include poor graft function, organ dysfunction, patient refusal, and 1 early death due to cerebral hemorrhage.

Among the patients who received at least 1 cycle of azacitidine, 2 patients suffered relapses; 1 of these patients was receiving 16 mg/m^2 and 1 was receiving 24 mg/m^2. A total of 4 patients have suffered relapse after completion of azacitidine treatment. Twice as many relapses have occurred in patients who were not receiving azacitidine. Thus at this point, with a median follow-up of 6 months, the actuarial 1-year event-free survival is approximately 60%, which is a promising interim analysis result in this population of

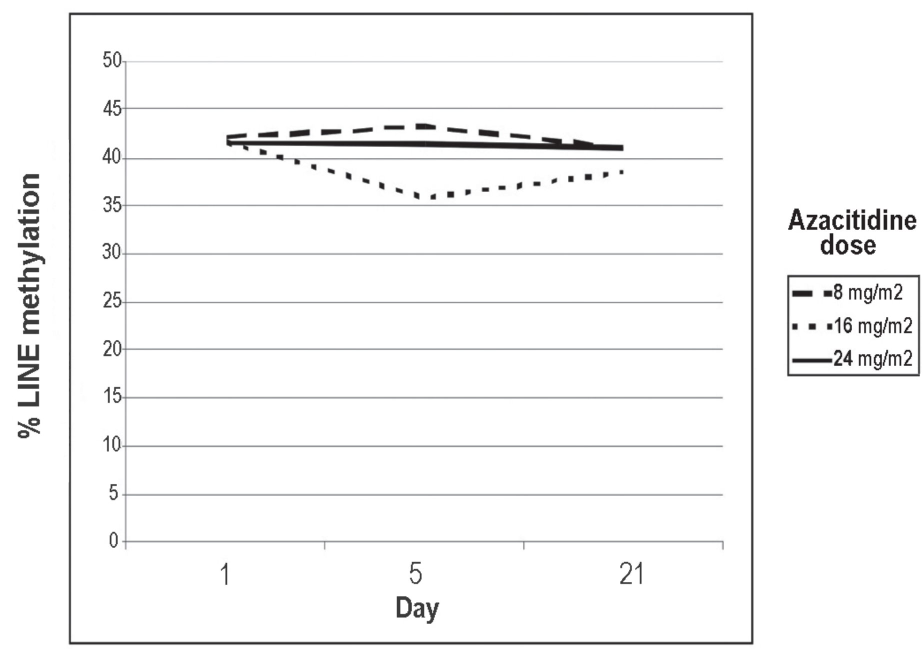

Figure 2. Illustration of possible azacitidine dose/schedule combinations selected by the method for the next patient.

Criteria for Eligibility to Receive Azacitidine Maintenance Therapy

Complete remission after transplantation
Serum creatinine <1.6 mg/dL
Serum bilirubin <1.6 mg/dL
Serum glutamic pyruvic transaminase <3 × upper limit of normal
Platelet count >15,000/mm^3
Absolute neutrophil count >1000/mm^3
No active bleeding
No uncontrolled acute GVHD
No acute GVHD grade III or IV
No life-threatening infection

patients and suggests that we are pushing these relapses later and later, but this result must be proven over time.

The LINE global methylation assay, although it is not built into the statistical design, may enable monitoring of a molecular surrogate marker for DNA methylation. Thus far, at azacitidine doses up to 24 mg/m^2, we have not seen changes before and after administration of the drug. This trial may be the first to use this tool to assess treatment response in bone marrow transplantation patients. If we continue to see no change over time, it may be because this global methylation assay is not a useful surrogate marker.

Thus far no patients have suffered serious adverse effects from azacitidine treatment. Some patients have shown increased transaminase, and there has been one possible serious drug interaction in a patient who received pentamidine, voraconazole, and azacitidine on the same day. There were some cases of hematologic toxicity, but these were minor.

Conclusions

In this study group, a heavily pretreated cohort, 60% of the patients received azacitidine, indicating that in a healthier patient population a higher percentage of patients would be treatable. As of now, we know for sure that we can deliver up to 4 cycles at 32 mg/m^2. Ultimately it will be necessary to study patients receiving 1 to 2 years of therapy, but currently we do not have the logistics or the manpower to organize a long-term investigation, although we are treating 5 patients off protocol who have been receiving the drug for up to 2 years without major side effects. When patients return home, it is necessary to negotiate with the institutional review board to arrange for ongoing drug administration. Because we cannot undertake such an endeavor at this time, we are attempting to demonstrate that azacitidine can be administered in the early posttransplantation period, when the risks for myelosuppression and other adverse events such as GVHD are high. Our successful results suggest that it also can be administered for a long time.

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Maximizing Treatment Outcomes for MDS in the Transplant Patient

CME Assessment Test

1. To date, most retrospective studies have shown that an advantage of using Reduced-Intensity Conditioning in Patients with MDS is:
A. Reduced relapse rates
B. Reduced non-relapse mortality
C. Superior overall survival
D. None of the Above

2. Which of the following is true regarding timing of transplantation for treatment of MDS:
A. Determination of disease stage by use of a tool such as the IPSS is an important step in determining optimal transplantation timing.
B. In patients with low- or intermediate-1–risk disease, a delay in stem cell transplantation may lead to a gain in life expectancy.
C. In patients with high- or intermediate-2–risk disease, delay of stem cell transplantation may lead to a loss in life expectancy.
D. All of the above.

3. Based on the results of the study by Kuendge and colleagues, what treatment should be offered to MDS patients younger than 50 years with IPSS low-risk disease:
A. Immediate treatment with allogeneic stem cell transplantation.
B. Immediate treatment with AML-type chemotherapy.
C. A watch-and-wait policy or intervention with nontransplantation strategies until the disease progresses.
D. None of the above.

4. Which of the following are therapeutic effects of hypomethylating agents used to treat MDS:
A. An overall antimalignancy effect through inhibition of DNA methyltransferase.
B. Restores activity to tumor-suppressor genes.
C. Delaying the time to disease progression.
D. All of the above.

5. Which of the following are interim results for the study of azicitidine for posttransplantation treatment of AML and MDS:
A. A small number of patients suffered severe adverse effects from azacitidine.
B. After an initial period during which azacitidine was well tolerated, doses of azacitidine were increased.
C. The LINE global methylation assay clearly shows a change in DNA methylation in response to azacitidine in 50% of patients.
D. None of the above.

6. Which of the following is true of the WPSS:
A. It incorporates transfusion dependency as a variable.
B. Its development required major reclassification of histological markers of MDS>
C. Like the IPSS scoring system, it is not time dependent.
D. All of the above.

CME Assessment Test Answer Sheet – Program ID #08143

Release Date: May 1, 2008 Last Review Date: May 1, 2008 Expiration Date: May 1, 2009

Instructions

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

1.	A	B	C	D	**3.**	A	B	C	D	**5.**	A	B	C	D
2.	A	B	C	D	**4.**	A	B	C	D	**6.**	A	B	C	D

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

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

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

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

I have read these articles on Maximizing Treatment Outcomes for MDS in the Transplant Patient, published in *Blood and Marrow Transplantation Reviews*, and have answered the CME test questions and completed the Evaluation Form for this activity.

Signature Date

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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.

Eapen M, Rubinstein P, Zhang M-J, et al: Outcomes of transplantation of unrelated donor umbilical cord blood and bone marrow in children with acute leukaemia: a comparison study. *Lancet*. 2007;369:1947-1954.

In the absence of HLA-matched sibling marrow, allele-matched bone marrow is regarded as the graft source of choice for children requiring allogeneic hematopoietic stem cell transplantation. Umbilical cord blood is an increasingly available alternative in this situation. The outcomes of umbilical cord blood and bone marrow transplantation were compared in children with acute leukemia, including assessment of the effects of cell dose and HLA matching.

The study used U.S. registry data on 503 children (younger than age 16) with acute leukemia who underwent umbilical cord blood transplantation and 282 who underwent bone marrow transplantation. The cord blood transplants were matched in 35 cases, HLA-mismatched for one antigen in 201 cases, and mismatched for two antigens in 267 cases. The bone marrow transplants were matched in 116 cases and mismatched in 166. Five-year leukemia-free survival was compared between the cord blood and bone marrow transplant groups.

For children receiving cord blood transplants mismatched for one or two antigens, 5-year leukemia-free survival was similar to that of children receiving matched bone marrow transplants. For children receiving matched cord blood, survival may have been higher than with matched bone marrow. Two-antigen-mismatched umbilical cord blood was associated with an increased risk of transplant-related death, relative risk 2.31. A similar increase may have been present for children receiving one-antigen-mismatched cord blood and a low cell dose. Two-antigen-mismatched cord blood transplants were associated with reduced relapse rates.

The results support the use of one- or two-antigen mismatched cord blood transplants for children with acute leukemia. The risk of transplant-related death after cord blood transplantation is decreased at higher levels of HLA matching and higher cell doses. These considerations warrant further investment in large-scale cord blood banking to increase HLA diversity.

O'Shaughnessey MJ, Chen Z-M, Gramaglia I, et al: Elevation of intracellular cyclic AMP in alloreactive CD4+ T cells induces alloantigen-specific tolerance that can prevent GVHD lethality in vivo. *Biol Blood Marrow Transpl*. 2007;13:530-542.

Elevated cyclic AMP (cAMP) levels have been linked to increased proliferation of some cell types, including epithelial cells and hepatocytes; but inhibited proliferation of other types, including smooth muscle, neuronal, and lymphoid cells. In T lymphocytes, cAMP serves as an important negative regulator—in vitro studies have shown that high cAMP levels are associated with T cell hyporesponsiveness. A technique of elevating intracellular cAMP levels in alloreactive T cells during primary mixed lymphocyte reactions (MLRs) was investigated as a means of inducing alloantigen-specific tolerance and preventing graft-versus-host disease (GVHD).

Primary MLR cultures containing purified CD4+ T cells as responders and irradiation MHC class II disparate splenocytes as stimulators were treated with the ^{8}Br-cAMP, a cell-permeable cAMP analog; and isobutylmethylxanthine (IBMX), which prevents degradation of intracellular cAMP via inhibition of phosophodiesterases. The resulting increase in intracellular cAMP was associated with sharp reductions in T cell proliferation and interleukin-2 responsiveness. Viable T cells isolated on day 8 showed impaired responses to restimulation with alloantigen, yet no change in response to nonspecific mitogens.

In labeling experiments, cAMP/IBMX limited the number of cell divisions, thus inhibiting alloreactive T cell proliferation. This made the cells more susceptible to apoptosis, while reducing responsiveness to restimulation with alloantigen in nondeleted alloreactive T cells. In in vivo studies, CD4+ T cells treated with cAMP/IBMX had reduced capacity to induce lethal GVHD in MHC class II disparate bone marrow recipients. This was despite the fact that other CD4+ T cell responses remained intact.

These studies show that manipulations to increase intracellular cAMP in CD4+ T cells can induce long-term alloantigenen tolerance. In vivo, this tolerance appears adequate to inhibit GVHD while maintaining normal nonalloreactive T cell functions. The findings help to validate the concept of using cAMP-elevating pharmaceutical treatments for prevention and treatment of GVHD and other T cell-mediated immune disorders.

Galan-Caridad JM, Harel S, Arenzana TL, et al: Zfx controls the self-renewal of embryonic and hematopoietic stem cells. *Cell*. 2007;129:345-357.

The ability to self-renew in an undifferentiated state is a unique characteristic of stem cells. However, it remains unclear whether the mechanisms governing self-renewal are the same for pluripotent embryonic stem cells (ESCs) as for tissue-specific adult stem cells. A series of experiments were performed to evaluate the role of Zfx, a zinc finger protein of the highly conserved Zfy family, in stem cell function.

Conditional gene targeting studies were performed to assess the functions of Zfx in ESCs and adult hematopoietic stem cells (HSCs). In Zfx-deficient ESCs, self-renewal was impaired but differentiation was unchanged. In contrast, Zfx-overexpressing ESCs remained undifferentiated, which promoted self-renewal. Zfx was required for self-renewal of adult HSCs, although deletion of Zfx had no effect on erythromyeloid progenitor cells or fetal HSCs.

In both murine cell types, Zfx-deficient stem cells exhibited increased apoptosis along with cell-specific upregulation of stress-inducible genes. Target genes common to both ESCs and HSCs, including Tbx3 and Tcl1, were directly activated by Zfx. In addition, Zfx activated other genes specific to ESCs, including genes involved in stem cell self-renewal.

The results suggest that Zfx is a shared transcriptional regulator of both ESCs and adult HSCs in mice. Thus both pluripotent ESCs and adult tissue-specific HSCs appear to share the same molecular basis for their property of self renewal. Further studies may aid in understanding the mechanisms of self-renewal in various types of stem cells, including tumor-initiating cancer stem cells.

Bruno B, Rotta M, Patriarca F, et al: A comparison of allografting with autografting for newly diagnosed myeloma. *N Engl J Med*. 2007;356:1110-1120.

For patients under age 65 diagnosed with myeloma, the standard treatment has been high-dose chemotherapy with autologous hematopoietic cell transplantation for bone marrow rescue. However, recurrences are common, largely because of myeloma cells remaining after chemotherapy. Some studies have achieved lower relapse rates and longer-lasting remissions using allogeneic stem cell transplantation. This trial compared allografting with autografting for patients with newly diagnosed myeloma, with the presence or absence of an HLA-identical sibling used as the criterion for treatment assignment.

Over a 6-year period, 162 patients, aged 65 years or younger, with newly diagnosed stage II or III myeloma and at least one sibling were enrolled. All received induction chemotherapy with vincristine, doxorubicin, and dexamethasone, followed by melphalan and a standard hematopoietic stem cell autograft. Patients with an HLA-identical sibling proceeded to nonmyeloablative total body irradiation, followed by allografting with stem cells from the sibling. Patients with no HLA-identical sibling received two myeloablative doses of melphalan after the induction protocol, each followed by autologous stem cell rescue. Overall and event-free survival were assessed at a median follow-up of 45 months.

Median overall survival was 80 months for the patients with HLA-identical siblings, compared to 54 months for those without an available sibling allograft. Event-free survival was 35 and 29 months, respectively. Treatment-related mortality was similar for patients who completed their assigned treatment: 58 patients in the autograft-allograft group and 46 in the autograft-autograft group. However, disease-related mortality was lower among patients receiving allografts: 7%, compared with 43% for those receiving autografts only.

Grade II to IV graft-versus-host disease (GVHD) occurred in 43% of the autograft-allograft group, including a 4% rate of grade IV GVHD. At a median follow-up of 38 months, 38% of patients in the autograft-allograft group were in complete remission, whereas 54% of patients receiving double autografts had died.

When an HLA-identical sibling is available, stem cell allografting improves survival in patients with newly diagnosed myeloma, compared with double autografts. The authors report just 7 relapses among 32 patients who achieved complete remission, with follow-up of up to 7 years. The nonmyeloablative conditioning regimen used in the study may promote a graft-versus-myeloma effect without the development of GVHD.

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