Blood and Marrow TRANSPLANTATION

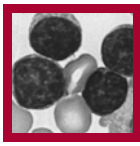
REVIEWS

A Publication of the American Society for Blood and Marrow Transplantation

Issues in Hematology, Oncology, and Immunology

VOLUME 15 NO 1 2005

IN THIS ISSUE



ASBMT™
American Society for Blood and Marrow Transplantation

Can't We All Get Past All the Bad Blood?

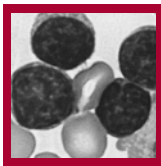
by John R. Wingard, Editor

Try as hard as it can, the myelodysplastic bone marrow just cannot seem to meet the ordinary day-to-day needs for blood cells. Indeed, failure is destined no matter what we do. Then, when it seems as bad as it can get, it only gets worse: total marrow shutdown or change into leukemia. Effective therapy has been long overdue.

The clonal nature of the myelodysplastic syndromes has been well accepted. Certain hematologic and cytogenetic characteristics have been identified as markers with important prognostic significance. However, beyond these descriptors, we have lacked knowledge of what are the biological underpinnings that determine the protean clinical manifestations and the highly variable clinical course of the various myelodysplastic syndromes.

This issue of *BMTR* contains a transcript of a symposium that addresses the topic of myelodysplastic syndromes, which was presented at the 2005 Tandem BMT Meetings in Keystone, Colorado. In the first presentation, Dr. John M. McCarty describes the current method of classifying the myelodysplastic syndromes, the utility of the international prognostic scoring system, a summary of the results of current therapies, the early promise of the recently introduced methyltransferase inhibitors, the role of stem cell transplantation, and an algorithm that suggests ways to incorporate the various treatment options into management of individual patients. In the second presentation, Dr. Margaret K. Yu describes the role of DNA methylation in health and neoplasia and addresses how the methyltransferase inhibitors can undo the silencing of tumor suppressor genes thought to play a contributing role in the myelodysplastic syndromes. Several avenues of future research are discussed.

Clearly, progress has been made in our understanding of the pathogenesis of the myelodysplastic syndromes. These insights are leading to new therapeutic approaches. These have not allowed us to put bad blood behind us yet, but there is a thaw in our heretofore strained relationship between disease, understanding, and intervention.



ASBMT™

American Society for Blood and Marrow Transplantation

Executive Office
American Society for Blood and Marrow Transplantation
85 West Algonquin Road, Suite 550
Arlington Heights, IL 60005-4425
(847) 427-0224; fax (847) 427-9656
e-mail: mail@asbmt.org

Publishing and Production Services
CJP Medical Communications, a division of Carden Jennings Publishing Co., Ltd.

Blood and Marrow Transplantation Reviews is published quarterly by CJP Medical Communications.
375 Greenbrier Dr., Suite 100, Charlottesville, VA 22901
phone (434) 817-2000; fax (434) 817-2020

This publication is supported by an unrestricted educational grant from Pharmion.

PRELIMINARY APPLICATION

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

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

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

Affiliate Membership is available to allied non-MD or non-PhD professionals who have an interest in blood and marrow transplantation. This category is especially appropriate for nursing and administrative staff of bone marrow transplant centers, collection centers, and processing laboratories, and for professional staff of corporations that provide products and services to the field of blood and marrow transplantation.

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.***

To become a member of ASBMT

copy and return this page with the required documentation and annual dues to:

ASBMT
85 West Algonquin Road, Suite 550
Arlington Heights, IL 60005

name _____ position _____

institution _____

address _____

city _____ state _____ zip/postal code _____ country _____

telephone number _____ fax number _____

email address _____

Membership:

☐ full $175 ☐ associate $175 ☐ affiliate $125 ☐ in-training $75

BMT Tandem Meetings Will Convene in Hawaii

Honolulu in February. It would be hard to think of a better time and location for the 2006 BMT Tandem Meetings—the annual meetings of ASBMT and the Center for International Blood and Marrow Transplant Research.

Scheduled for Feb. 16-20, the BMT Tandem Meetings will present the latest advances in the broad field of cellular therapy and blood and marrow transplantation, addressed in plenary sessions, concurrent sessions, workshops, poster sessions and symposia. In addition, about 60 original abstracts will be selected for oral presentation.

Housing and on-line registration will open in July. The abstract deadline is Oct. 3.

The topics for major sessions will be cancer vaccines and T-cell therapy, cell trafficking and homing, follicular lymphoma, genomic polymorphism, stem cell biology, aging and transplantation, cord blood transplants, genomics, Hodgkin's disease, immune reconstitution, infection response, myeloma/plasma cell disorders, nanotechnology, chronic GvHD, late effects, pediatric cancer, preclinical models, reduced-dose therapy and outcomes related to practice variables.

In addition to the general sessions, there will be conferences for specific transplant personnel:

- Transplant center medical directors, Feb. 19
- Transplant nurses, Feb. 18-20
- BMT pharmacists, Feb. 15-16
- Clinical research associates data managers, Feb 15-17
- Transplant center administrators, Feb. 17-18
- Physician fellows-in-training, Feb 15

The meetings will be at the Hawaii Convention Center and the headquarters at the Ala Moana Hotel, with additional accommodations at the Renaissance Ilikai Waikiki Hotel and the Hilton Hawaiian Village.

Online information and registration are at *www.asbmt.org*.

Nelson Chao Installed as ASBMT President

Nelson Chao, MD, professor of medicine and immunology and director of the bone marrow transplant program at Duke University Medical Center, has been installed as president of the American Society for Blood and Marrow Transplantation.

Robert Soiffer, MD, an associate professor of medicine at Harvard Medical School and chief of the Division of Hematologic Malignancies at Dana-Farber Cancer Institute, is the newly elected and installed vice president, to become president in 2007.

The installation of officers and directors occurred at the Tandem BMT Meetings in February in Keystone, Colo. The election was by mail ballot among members of the Society in January.

Newly elected and installed directors are:

- Stephanie Lee, MD, MPH, Dana-Farber Cancer Institute
- Scott Rowley, MD, Hackensack University Medical Center
- Marcel van den Brink, MD, PhD, Memorial Sloan-Kettering Cancer Center

Robert Negrin, MD, associate professor of medicine and director of the Division of Bone Marrow Transplantation at Stanford University, was elevated to president-elect, and will assume the presidency in 2006.

The new ASBMT president, Dr. Chao, is a graduate of Harvard University. He earned his medical degree at Yale University in 1981. He completed a residency in internal medicine at Stanford University Medical Center and continued his fellowship training at Stanford University. He stayed on as a faculty member until 1996 when he moved to Duke University.

Among his principal interests are graft-versus-host disease, both clinically as well as in animal models, and immune reconstitution and post-transplant immune therapy.

2005 Tandem BMT Meetings Audiocassettes Available

Audiocassettes can be purchased for all plenary and concurrent scientific sessions, workshops and oral abstract sessions held at the Tandem BMT Meetings this past February in Keystone.

Also available are presentations in the conferences of the transplant nurses, BMT pharmacists, clinical research associates and BMT center administrators.

Information and online purchase are at *www.CMEunlimited.org/bmt*.

Abstracts Posted Online

Abstracts presented at the 2005 Tandem BMT Meetings were published in the February 2005 issue of *Biology of Blood and Marrow Transplantation* (Vol. 11, No. 2, Supplement 1). They also can be viewed online at the ASBMT Web site *www.abstracts2view.com/bmt*.

ASBMT

Symposium Report

Blood and Marrow TRANSPLANTATION REVIEWS

Novel Therapies in the Treatment of Myelodysplastic Syndromes

Adapted from a CME symposium presented at the American Society for Blood and Marrow Transplantation and the Center for International Blood and Marrow Transplant Research 2005 Tandem BMT meetings, February 2005, Keystone, Colorado.
This program is supported by an unrestricted educational grant from the Pharmion Corporation.

MEDICAL COLLEGE OF WISCONSIN CME program

Faculty

John M. McCarty, MD

Medical Director, Bone Marrow Transplant Program, Massey Cancer Center, Virginia Commonwealth University Medical Center, Richmond, Virginia, USA

Margaret K. Yu, MD

Division of Medical Oncology, Huntsman Cancer Institute, University of Utah School of Medicine, Salt Lake City, Utah, USA

Faculty Disclosure

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

John M. McCarty, MD, has no relationships to disclose.

Margaret K. Yu, MD, has no relationships to disclose.

Continuing Medical Education Credit

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

The Medical College of Wisconsin designates this educational activity for a maximum of 1.0 category 1 credits toward the AMA Physician's Recognition Award. Each physician should claim only those credits that he/she actually spent in the activity. Credit is only available to those physicians who did not receive credit for attending the live program February 10, 2005.

Target Audience

This CME activity is designed for physicians who are hematology and oncology specialists involved in BMT and those interested in treating myelodysplastic syndromes.

Objectives

On completion of this activity the participant will be able to:

- Describe myelodysplastic syndromes (MDS) by prognosis and pathologic features.
- Discuss the goals of therapy for MDS including preparatory treatments for patients eligible for BMT.
- Understand the mechanism by which DNA methylation inhibitors may reactivate tumor suppressor genes previously silenced by DNA methylation.
- Characterize our current ability to target DNA methylation and other gene silencing processes into new therapies.

Overview

This symposium report reviews the biological and clinical aspects of the myelodysplastic syndromes (MDS), including current therapies, new treatment options, and emerging therapies, in particular the methyltransferase inhibitors. MDS represents a group of clonal disorders in which the expansion of a pathologic putative stem cell results in ineffective hematopoiesis of one or more cell lineages. These disorders are associated with both qualitative and quantitative abnormalities of the major cell lines of the blood, and inevitably progress to marrow failure or acute leukemia. The protean presentation of MDS contributes to an often confusing overlap with other hypo- and hyperproliferative hematologic disorders such as aplastic anemia, the myeloproliferative syndromes, and de novo acute leukemias. Optimal management of MDS requires a clear understanding not only of the clinical features of these heterogeneous disorders, but also of the prognostic value of the biologic presentation in each individual patient to allow an optimal patient and disease-tailored clinical approach. Whereas previously for most patients the best supportive care consisted of transfusion and growth factor support alone, newer approaches can now offer more effective palliative and even curative treatments to a greater pool of affected patients. This presentation will review diagnosis and prognostic scoring systems and will explore newly available therapies as well as those under development, which have become available due to a better biologic understanding of MDS.

Myelodysplastic Syndromes: Principles, Practice, and State-of-the-Art

John McCarty, MD

The myelodysplastic syndromes (MDS) are a heterogeneous group of clonal stem cell disorders characterized by hypercellular marrow, peripheral cytopenias, ineffective hematopoeisis, and cellular functional abnormalities. These diseases have a highly variable natural history. They occur mostly in elderly patients, but their presentation is as heterogeneous as the patients who contract them. Unless stem-cell-based therapy such as allogeneic transplantation effects permanent control, death due to marrow failure, with or without or conversion to acute myelogenous leukemia (AML), will ensue.

MDS Diagnosis and Classification

One of the difficulties of MDS diagnosis is its significant clinical overlap with a variety of other disorders, particularly AML. Hypoplastic MDS is often very difficult to differentiate from aplastic anemia. Many of the myeloproliferative disorders will have a crossover such as chronic myelomonocytic leukemia (CMML), large granular leukemia, or other autoimmune diseases that also may have a component of dysplasia that must be differentiated from myelodysplasia. When a definite diagnosis of MDS is made, characterization of disease stage and severity are important in planning treatment, so several systems of MDS classification have been developed.

French-American-British and World Health Organization Classification

The French-American-British (FAB) classification was the first systematic nomenclature for the classification of MDS. FAB classification identifies 5 morphologic subtypes of MDS: refractory anemia (RA), RA with ringed sideroblasts (RARS), RA with excess blasts (RAEB), and RAEB in transformation (RAEB-T). The FAB classification system very clearly correlates with overall survival, with patients with earlier forms doing better than those with AML or more advanced disease (Figure 1).

Recently the World Health Organization (WHO) modified the FAB system on the basis of the recognition that there are subsets of patients whose disease characteristics do not fit these criteria, and therefore some other subclassifications have been added, 5-q syndrome and unclassified MDS, defined by single-lineage nonerythriod dysplasia. In addition, the 3 FAB subtypes, RA, RARS, and RAEB, have been more specifically classified into subtypes based on characteristics such as presence or absence of dysplasia and percentages of blasts and ringed sideroblasts (Table 1).

International Prognostic Scoring System

Despite its usefulness, the WHO scoring system does not always correlate with disease natural history in MDS, so that that within the same classifications some patients do better than others. Greenberg et al [1] formulated the International Prognostic Scoring System (IPSS) to identify patients with similar outcomes based on nonmorphologic risk factors such as cytogenetics, percentage of bone marrow blasts, and number of distinct lineage cytopenias. The IPSS system was developed on the basis of outcomes for 816 patients from large institutional national trials with de novo MDS (excluding CMML patients). IPSS scores are assigned for different prognostic variables including percentage of marrow blasts, karyotype (good, intermediate or poor), and number of cytopenias. The MDS subtype known as 5q- syndrome, which is associated with an isolated del(5q) cytogenetic abnormality and is associated with a long survival, is included in the good karyotype classification.

These scores are added together to determine the patient risk status (Table 2). Greenberg et al showed that in terms of survival the IPSS score correlated very well with natural history such as median survival and time to AML transformation.

These improved systems for understanding MDS natural history, determining prognosis, and predicting patient outcomes are important tools for implementing optimal treatment strategies.

MDS Treatment

Transplantation

Bone marrow transplantation (BMT) is currently the only known curative treatment for MDS. But given that the largest group of patients who develop myelodysplasia are well above the age of 60 years, only a subset of patients are BMT candidates. When only conventional allogeneic transplantation is taken into consideration, less than 5% of patients with myelodysplasia are candidates for stem cell transplantation. However transplantation has changed from the time that this estimate was made, so it is important to look at current technologies and individualize therapy according to risk group, patient preference, donor availability, and patient candidacy, which is based only in part on age.

Best Supportive Care

If curative BMT is not an option, then the gold standard for MDS treatment is best supportive care, which includes growth factors and transfusion support while aiming to decrease transfusion needs, reduce the risk of infection, and increase the quality of life for these patients.

Anemia is very prevalent in MDS patients, and the majority require transfusions. MDS patients may benefit from treatment with erythropoietin (EPO). If patients are treated with erythropoeitic agents, iron support is critical, and response is usually seen within 1 to 2 months. Weekly dosing is not indicated;

Table 1. WHO Modifications of FAB MDS Subtypes*

Classification	Category	Peripheral Blood Morphology	Bone Marrow Morphology
1a	RA without dysplasia	< 1% blasts, <1000/mm3 monocytes	<5% blasts, <15% ringed sideroblasts
1b	RA with dysplasia	<1% blasts, <1000/mm3 monocytes with dysplastic lineage +/- giant platelets	<5% blasts, <15% ringed sideroblasts with dysplastic myeloid and/or megakaryocytes
2a	RARS without dysplasia	< 1% blasts, <1000/mm3 monocytes	<5% blasts, >15% ringed sideroblasts
2b	RARS with dysplasia	<1% blasts, <1000/mm3 monocytes with dysplastic lineage +/- giant platelets	<5% blasts, >15% ringed sideroblasts with dysplastic myeloid and/or megakaryocytes
3a	RAEB I	1%-5% blasts, <1000/mm3 monocytes	5%-10% blasts
3b	RAEB II	6%-20% blasts, <1000/mm3 monocytes	11%-20% blasts
4	CMML	1%-20% blasts, >1000/mm3 monocytes	0%-20% blasts

*In the WHO system, the FAB subtypes RA, RARS, and RAEB are further characterized on the basis of presence or absence of dysplasia and percentages of blasts and ringed sideroblasts.

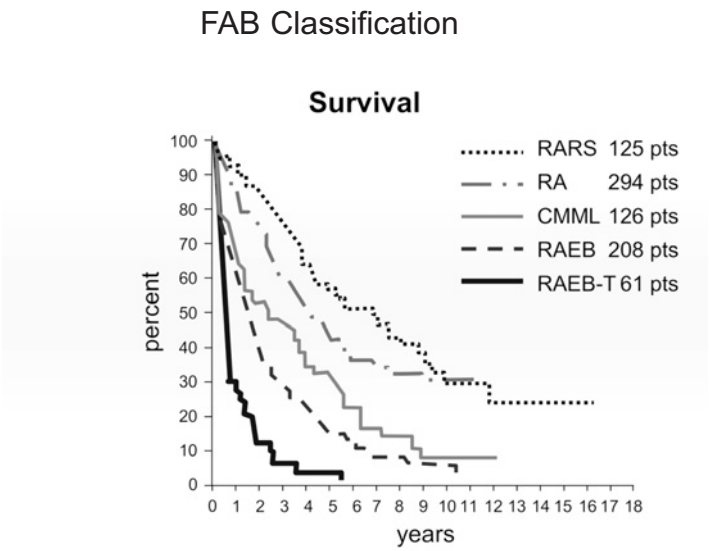


Figure 1. Patient survival based on FAB classification. Reprinted with permission from [1].

patients very often do well with lower dosing. In patients who either do not respond to EPO within a 1- to 2-month period or initially respond and then fail to respond, a synergistic effect may be obtained by the addition of low-dose granulocyte colony-stimulating factor (G-CSF), sometimes at a dose as low as 1 to 2 μg/kg. This effect is most often seen in patients with the lowest EPO levels. Growth factor support has not made a major impact on overall survival in MDS, however.

Neutropenia is seen in 50% of patients, and 30% to 35% of these patients have an absolute neutrophil count under 1000. Only 10% of these patients have infections, however, so the use of routine antibacterial prophylaxis is not indicated. Thrombocytopenia affects 25% to 50% of patients, but thrombopoietic agents have had no significant impact on transfusion needs in MDS patients.

Chemotherapy

A multiplicity of studies have shown that MDS patients do not benefit from low-dose cytarabine or other forms of chemotherapy such as Mylotarg. AML-type induction chemotherapy may play a role in treatment of younger patients with normal cytogenetics without a prior history of abnormal blood count. There may be a role for chemotherapy in patients who will undergo autologous stem cell transplantation or who may be candidates for reduced-intensity transplantation. Other patients who are not transplantation candidates should be referred to clinical trials or considered for some of the newer agents.

New Treatment Agents

Antithymocyte globulin (ATG) has been used for some time in treatment of MDS and other diseases that have an immune component to their cytopenias. Characteristics of patients most likely to respond to ATG include HLA-DR-15 positivity, presence of a PNH clone indicated by flow cytometry, age younger than 70 years, and a brief transfusion history.

Thalidomide has also been proposed as a therapy for MDS patients. Theoretically it targets the neoangiogenesis observed in the bone marrow in MDS and it may also change the cytokine milieu and signaling characteristics associated with the ineffective hematopoeisis seen in myelodysplasia. Supportive evidence comes from 3 studies in which a 20% response rate was noted in a total of 142 patients. However, in a study from the Mayo clinic using higher doses of thalidomide, the response rate was actually inferior. Thalidomide is very poorly tolerated by many patients, particularly the elderly.

Exciting data have recently been reported by List et al [2] for CC5013 (lenalidomide), an immunomodulatory drug that is chemically similar to thalidomide. Erythroid responses were seen in 64% of patients treated with CC5013, including 20 of the 33 who had either a transfusion independent major response or an increase in hemoglobin of greater than 2 g/dL. Major cytogenetic responses seen with CC5013 were also very exciting because they indicated that it may actually improve karyotypic abnormalities. Complete cytogenetics responses were seen in 59% of patients, and nearly 65% had a greater than 50% decrease in their abnormal karyotypes. Red blood cell responses showed some durability, with more than a 38-week median response time. The main side effect of CC5013 is clinically significant myelosuppression. Patients most likely to benefit are those with a low-intermediate-1 IPSS score who had failed prior therapy or with refractory anemia with less than 5% blasts. Patients with 5q-syndrome demonstrated an even higher response rate to CC5013.

Methyltransferase Inhibitors

A drug that has recently been FDA approved and is in current use for MDS treatment is the methyltransferase inhibitor 5-azacitidine, trade name Vidaza (azacitidine). The pivotal trial that supported its approval was Cancer and Leukemia Group B 9221 by

Table 2. International Prognostic Scoring System

A. Scoring System					
Prognostic Variables					
% Marrow blasts	<5	5-10		11-20	21-30
Karyotype*	Good	Intermediate	Poor		
Cytopenia	0/1	2/3			
Score	0	0.5	1.0	1.5	2.0
*Good, normal or -5q, -Y, -20q; intermediate, other; poor, -7, complex.					
B. Risk Classification					
Low	0				
Intermediate 1	0.5-1.0				
Intermediate 2	1.5-2.0				
High	≥2.5				

Silverman et al reported in *J Clin Oncol* [3]. Patients with clinical myelodysplasias from RA to AML and CMML were stratified and randomized either to best supportive care or to azacitidine given as 75 mg/m^2 subcutaneously for 7 days every 28 days for a minimum of 4 cycles. Patients on supportive care who met progression criteria were crossed over to the azacitidine arm. Quality of life and bone marrow evaluations were performed at 2-month intervals. Analysis of response was impressive. In addition to a 7% complete response rate, the total response rate was more than 60% compared to 5% in the best supportive care arm (Table 3). Time to AML was greatly improved in patients receiving azacitidine, with a more than 9-month gain in time, and the percentage of patients who relapsed with AML presentation decreased from 38% for patients receiving supportive care to 15% for patients receiving azacitidine. These results were all statistically significant. Survival benefit was independent of FAB classification; azacitidine was superior to supportive care in low- and high-risk disease. It is also important to note that quality of life was also significantly improved over best supportive care in patients receiving the azacitidine.

Azacitidine is fairly well tolerated, and the safety profile has been well characterized. The most common adverse event is an injection-site reaction, which responds either to topical steroids or antihistamines. Other common adverse events are consistent with its known pharmacology. Approximately 60% of patients treated with azacitidine developed neutropenia. That incidence is highest in the first cycle and decreases in responders in subsequent cycles. Gastrointestinal side effects including nausea may occur and can be treated by pretreatment with antiemetic medications. During the first treatment cycle, some patients who develop neutropenia will also suffer worsening anemia and thrombocytopenia, but in those responding, these cytopenias will improve with subsequent dosing.

Given the fact that this drug has been shown to be effective in terms of overall survival and delay of the time to onset of AML, azacitidine may be considered as our new standard of best supportive care. These data also suggest that this drug should be used earlier in the treatment course, and not after failure of transfusion/growth factor support.

Another methyltransferase inhibitor, decitabine, has also been studied and has shown promise. Issa et al [4] looked at low-dose decitabine treatment in 44 patients. In 11 of 18 patients who responded, the median duration of remission was approximately 8 weeks. Very significant myelosuppression was a side effect: the mean time to recovery to greater than 1000 was approximately 45 days for granulocytes and 39 days for platelets greater than 20,000. In remission the mean time to granulocyte recovery was almost 36 days. In another study of 66 patients with high-risk MDS treated with decitabine [5], there were 34 responses overall; 13 patients had a complete response and 9 had a trilineage improvement. Again delayed cytopenia was the main adverse feature, particularly in the first treatment cycle, resulting in an almost 8% mortality. Median duration of the response was approximately 31 weeks, but interestingly, 16 of the 50 patients with clonal abnormalities developed a cytogenetic complete remission (CR) that was relatively durable, lasting a median of 7.5 months.

Table 3. Analysis of Response to Azacitidine*

	Supportive Care (n = 92)	Azacitidine (n = 99)	Crossover (n = 49)
Complete response	0 (0%)	7 (7%)†	5 (10%)
Partial response	0 (0%)	15 (16%)‡	2 (4%)
Improved	5 (5%)	38 (37%)‡	16 (36%)
Total	5 (5%)	60 (60%)‡	23 (47%)

*Study of the Cancer and Leukemia Group B [3].
†$P < .01$.
‡$P < .001$.

Considerations for Treatment Optimization

As transplantation physicians we are aware that long-term disease cure in MDS will require treatment directed at the stem cell. Relapse is always based in ineffective treatment of the stem cell clone. If we avoid transplantation we know that the disease course will lead to patient death. If we do use stem cell directed therapy, we may actually cure a considerable percentage of our patients. Initially there is significant risk to transplantation, however, and this risk makes the treatment decision difficult.

This difficult decision-making process can be facilitated in myelodysplasia patients by looking at the factors that determine the disease risk and perhaps increase risk in more advanced MDS. Less aggressive pretransplantation disease control increases the risk of relapse later. Higher IPSS scores going into transplantation, poor-risk cytogenetics, elevated myeloblast count, or longer pretherapy disease course before transplantation is attempted will all increase risk of disease relapse but are also areas in which risk can be modulated.

Ways to reduce therapy-related risks include the possibility of reducing the myelodysplastic stage prior to transplantation, looking at and accounting for comorbidities, treating patients with lower IPSS scores, sequencing transplantation earlier in the disease course, assessing patient performance status, and looking at specific stem cell sources and preparative regimens.

Transplantation survival in MDS patients is still based on FAB/WHO score. Because transplantation is clearly less successful with more advanced disease, the decision of when patients should undergo transplantation is critical.

A decision analysis by Cutler et al [6] looked at the years of life saved in MDS patients who undergo transplantation immediately:2 years from the time of consultation and diagnosis, or at the time of progression. In patients with low or intermediate-1 IPSS scores, years of life saved were actually maximized if transplantation was held off until patients showed evidence of disease progression. However, in high-risk patients with higher IPSS scores transplantation at the time of diagnosis was more favorable. The effect of recipient age, as is seen with any number of diseases for which transplantation is an option, is that older age is associated with poorer outcome.

Selection of an appropriate preparative regimen may confer an advantage. Witherspoon et al [7] looked at standard total-body irradiation regimens compared to weight-based busulfan and cyclophosphamide regimens versus the targeted busulfan and cyclophosphamide regimens and found an advantage to targeted busulfan regimens with these patients. Even with this optimal preparative regimen, IPSS score is still important both in

terms of the risk of relapse and the potential for relapse-free survival.

Additional risk factors have been seen in a number of other studies. Cytogenetics are a clear risk factor for relapse after transplantation. Those with complex cytogenetics have an 83% chance of relapse at 7 years. Associated with the IPSS cytogenetic risk, event-free survival (EFS) can range from 51% with a relapse of 19% in patients with good risk karytypes to an EFS of 6% with a relapse of 82% in patients with complex cytogenetics abnormalities. G-CSF-mobilized peripheral blood stem cells may actually reduce the risk of relapse, but at the expense of more prevalent chronic graft-versus-host disease. Transplantation-related mortality may be reduced as a result of faster engraftment, which has been well described in a number of other treatment modalities.

If transplantation is chosen as a treatment option, what are the expected outcomes? In looking at conventional matched-related (MRD) stem cell transplantation in patients at median age in the 30s and 40s, 7-year event-free survival is approximately 30% to 40% with better results seen using targeted busulfan preparative regimens. Relapse rate remains relatively high (20-40%), and the transplantation-related mortality is also problematic (30-40%), reflecting the older age of some of these patients. Conventional unrelated stem cell (MUD) transplantation was associated with higher transplantation mortality but lower relapse rates, which is expected with the use of unrelated or alternative donors or with high risk patients who were not in complete remission or had not attained stable disease prior to coming to transplantation.

Reduced intensity MRD and MUD transplants have been shown to be effective in a number of studies with EFS at 1 year of 60% and at 3 years of 30%, and modest transplantation-related mortality (30%). The intensity of the preparative regimen was important, with more intense preparations resulting in lower relapse rates (30% versus 60%) and better overall survival (OS). In these studies, better OS/EFS was associated with lower IPSS scores or attaining a CR/stable disease prior to transplantation.

Autologous stem cell transplantation may be a relatively underused resource for patients with myelodysplasia. It is feasible in patients who achieved a CR after induction chemotherapy. Polyclonal primitive progenitors can be mobilized after induction chemotherapy in patients with high-risk myelodysplasia, also making this a feasible approach. Data indicate that patients who achieved a complete remission and underwent autologous transplantation had a disease-free survival rate of about 33% with a relapse of 64%. The greatest benefit was seen in younger patients, with a 39% versus 25% survival in patients younger than 40 years. Comparable 4-year event-free survival has been obtained for induction chemotherapy followed by either matched-related transplantation or autologous stem cell transplantation. To summarize study results, EFS at 2 to 4 years ranged between 23% and 34%, OS at 2 to 4 years was 39%, and the relapse rate remained in the 50% range. But there clearly is a patient population of patients that may benefit from this approach.

The use of pretransplantation cytoreductive chemotherapy is still controversial but may be beneficial. EFS was increased in patients with RAEB with greater than 10% blasts or RAEB-T who were pretreated compared with those who had not received induction chemotherapy, but relapse rates were comparable.

Induction chemotherapy to a complete remission is essential in patients receiving an autologous transplant to have appropriately prepared bone marrow for stem cell products for that transplant. In addition, studies suggest that induction chemotherapy to complete remission does improve survival in patients receiving reduced-intensity transplants, and it may allow more effective time for the graft-versus-MDS effect to be effectively established. The question remains whether induction chemotherapy increases transplantation-related mortality and morbidity to counteract these benefits.

Methyltransferase Inhibitors Combined with Transplantation

Methyltransferase inhibitors may be useful in reducing transplantation-related mortality and morbidity. We have seen that these drugs are less toxic than standard chemotherapy agents, therefore patients pretreated with methyltransferase inhibitors may come to transplantation with better performance status. In addition, because these drugs very clearly improve ineffective hematopoiesis and may normalize marrow cellularity, they may enable patients with hypoplastic MDS to have a more effective autologous stem cell harvest. The delay in onset of AML progression may allow an earlier and more effective graft-versus-MDS effect and perhaps reduce the relapse rate. A longer pretreatment course may allow transplant centers more time to organize the transplantation logistics and identify optimal donors.

We have recently started a small pilot study looking at patients who were treated with azacitidine as an intention-to-treat prior to transplantation. Ten patients, all referred to us with intermediate-2 or high IPSS score, received a minimum 4 cycles of azacitidine according to the CALGB 9221 study. Bone marrows were evaluated after every 2 cycles, and patients showing signs of progression received FLAG (fludarabine, cytarabine, and G-CSF) chemotherapy as induction and proceeded with best available transplant. Six of 10 patients achieved a CR or a very good partial response; 4 of 10 had disease progression and received FLAG chemotherapy. Of the 6 who responded to azacitidine, 2 were hypoplastic and were being considered for autologous stem cell transplantation. One of these patients, however, was found to have anthracycline related cardiomyopathy, making him ineligible for transplantation. The second patient died of a severe fungal infection. Of the remaining 4 FLAG patients, 2 underwent transplantation, and 2 were either ineligible or declined.

The 4 patients who received transplants after treatment with azacitidine included a 62-year-old white female who underwent a reduced-intensity MRD transplantation and at day +750 was doing well except for some mild mucosal graft-versus-host disease. A 19-year-old Indian male who received a conventional MUD transplant had late graft-versus host disease of the gut that had resolved at +600 days. We were able to find a donor for this patient because of the ability to treat him for 6 months with azacitidine. A 45-year-old African-American female was doing well with no treatment-related disease at day +125 after a conventional MRD transplantation. A 53-year-old white male with RAEBII had no pathological evidence of disease at day +95 after reduced-intensity MUD transplantation. Other patients in this study were too early to evaluate at the time of this report, but all patients had engrafted by day +15, and all patients had 100% donor chimerism at day 30. When we looked at how the FLAG patients fared, only 1 of 4 patients remained alive and this patient, who did not receive a

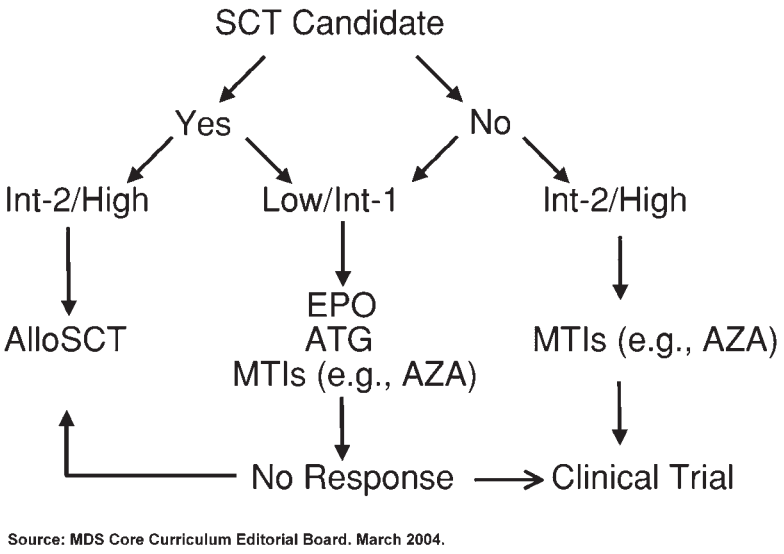


Figure 2. Myelodysplastic syndrome treatment algorithm.

transplant, had recently relapsed at 1 year. Although the numbers in this pilot study are small it is intriguing to see such differences in transplantation outcomes in patients attaining CR from either azacitidine or FLAG.

Conclusion

Figure 2 presents an algorithm for determining the best treatment approach for MDS patients. With low-risk patients stem cell transplantation may be an option, given the very good outcome for early-disease patients who are young and who have a very well-matched donor, but these patients may be counseled, should they choose to delay transplantation, that transplantation should be performed at the onset of any worsening of disease characteristics. Perhaps the use of some of the methyltransferase inhibitors, CC5013, or other very promising agents as a bridge prior to transplantation should be studied as alternatives to induction chemotherapy. Reduced-intensity transplantation or autologous transplantation may be considered in appropriate patients attaining a complete remission, and patients with high-risk disease should have access to this life-saving and potentially curative option. We have a number of novel agents that are showing great promise and that give MDS patients new hope for meaningful disease control as well as additional time with good quality.

We have seen some of the parameters that are important for diagnosis and risk assessments that help us counsel our patients. Improved biological understanding makes it possible to plan more individualized therapy. The transplantation physician should be involved relatively early in the treatment process to initially assess what transplantation options may be possible, taking into consideration all factors in addition to patient age. Induction chemotherapy alone has a very limited role to play in MDS treatment. Stem cell transplantation remains the only curative option, but reduced-intensity transplantation should remain investigational and should be part of ongoing clinical trials. We should look at these different treatment options as another way of tailoring treatments to a patient's disease status. We have a number of promising agents that are either available or on the horizon. At this time the methyltransferase inhibitors are the only agents that have clearly been shown to be superior to best supportive care, have changed the natural history of MDS, and may change the management paradigm that we have used up until now to treat MDS.

References

1. Greenberg P, Cox C, LeBeau MM, et al. International scoring system for evaluating prognosis in myelodysplastic syndromes. Blood. 1997;89:2079-2088.
2. List AF, Kurtin S, Glinsmann-Gibson B, et al. Efficacy and safety of CC5013 for treatment of anemia in patients with myelodysplastic syndromes (MDS). *N Eng J Med*. 2005;352:549-557.
3. Silverman LR, Demakos EP, Peterson BL, et al. Randomized controlled trial of azacitidine in patients with the myelodysplastic syndrome: a study of the Cancer and Leukemia Group B. *J Clin Oncol*. 2002;20:2429-2440.
4. Issa JP, Garcia-Manero G, Giles FJ, et al. Phase 1 study of low-dose prolonged exposure schedules of the hypomethylating agent 5-aza-2'-deoxycytidine (decitabine) in hematopoietic malignancies. *Blood*. 2004;103:1635-1640.
5. Wijermans P, Lubbert M, Verhoef G, et al. Low-dose 5-aza-2'-deoxycytidine, a DNA hypomethylating agent, for the treatment of high-risk myelodysplastic syndrome: a multicenter phase II study in elderly patients. *J Clin Oncol*. 2000;18:956-962.
6. Cutler CS, Lee SJ, Greenberg P, et al. A decision analysis of allogeneic bone marrow transplantation for the myelodysplastic syndromes: delayed transplantation for low-risk myelodysplasia is associated with improved outcome. *Blood*. 2004;104:579-585. Epub 2004 Mar 2.
7. Witherspoon RP, Deeg HJ, Storer B, Anasetti C, Storb R, Appelbaum FR. Hematopoietic stem-cell transplantation for treatment-related leukemia or myelodysplasia. *J Clin Oncol*. 2001;19(8):2134-2141.

Modulation of DNA Methylation

Margaret K. Yu, MD

Methylation of DNA modulates gene expression during development, regulates transcription, normal X-chromosome inactivation, and varying tissue-specific expression of particular genes. In malignancies, DNA methylation can also silence genes that help regulate cell-cycle progression and programmed cell death. Unlike gene inactivation by mutations or deletions, gene expression silenced by DNA methylation can be reversed in replicating cells by treatment with a DNA methyltransferase inhibitor. The DNA methylation inhibitor, azacitidine (Vidaza), has been approved for the treatment of patients with myelodysplastic syndrome (MDS). Azacitidine has been an effective therapy even in the treatment of patients with MDS at the French-American-British stages RARS and RAEB with high IPSS.

DNA Methylation in Normal Cells

DNA methylation plays an important role in fetal development in embryogenesis, in which genomic imprinting is done early in the first trimester of fetal development, and in X-chromosome inactivation. DNA methylation is also a mechanism by which tissues specifically express various genes and is a regulator of transcription. In eukaryotes and higher organisms, cytosines are modified by methylation. This process can occur anywhere in the genome: in the introns, the axons, and the gene promoters. The focal methylation of gene promoters is thought to repress suppression or "silence" specific genes. Approximately 5% of all the cytosines in the genome are methylated in normal cells.

DNA Methylation in Cancer Cells

Compared to normal cells, cancer cells have a lower level of global DNA methylation, but the methylation that occurs in cancer cells silences tumor suppressors. Individual cancers likely have several and may be hundreds of methylated genes, and individual tumor types have characteristic methylation profiles. Determination of methylation profiles for specific cancers is an active area of investigation. p16 is a cyclin kinase inhibitor that is commonly found to be methylated in melanoma, colon cancers, breast cancers, lung cancers, and MDS [1]. p15 is predominantly methylated in approximately 70% of all acute myelogenous leukemias and in MDS [2]. p15 has been found to be methylated in patients who have MDS with high IPSS scores and RAEB or RAEB-T FAB classification—now classified as AML. Other genes that have been identified to be methylated in MDS are calcitonin, E-cadherin, a gene that is important for metastasis and invasion, HIC-1, which is hypermthylated in cancer, and the estrogen receptor.

Reactivation of Tumor Suppressor Genes by DNA Methylation Inhibitors

Normally, CpG islands from promoters are available for docking by transcriptional machinery, and tumor suppressor genes are actively transcribed (Figure 1). In cancer cells, for some reason, the CpG islands in the promoter become methylated. The gene is still present in the genome but is now inactive and silent. The difference between DNA methylation and mutation or deletion of a specific gene is that this methylation can be reversed in replicating cells by treatment with a DNA methyltransferase inhibitor. Once the DNA methyltransferase inhibitor has been incorporated into the cell, active transcription resumes.

Theoretically, reexpression of a gene after demethylation by a methyltransferase inhibitor should result in a functional protein, but unfortunately in some cases, an active transcript does not result in a functional protein (Figure 2). Decitabine (5-aza-2'-deoxycytidine) is one of the DNA methyltransferase inhibitors that is available for clinical use [2]. Cells that have been treated with decitabine are globally more hypomethylated. However, this hypomethylation does not translate to an increase in the transcript numbers compared to control cells or reexpression of the protein as indicated by a Western blot results. In the normal genome there is diffuse methylation of various cytosines, and in the tumor DNA a lot of the cytosines are hypomethylated, with some focal hypermethylation of the promoters of tumor suppressor genes. Methylation fits into the Knudsen hypothesis in that two alleles must be inactivated for a tumor suppressor to be rendered completely dysfunctional. Therefore if one of the alleles sustains a hit, the heterozygous state is still able to carry on the normal tumor suppression function of the gene, and it is only after the second hit that the tumor suppressor is no longer functional. Inactivation of the second allele by methylation may occur in addition to the inactivation by

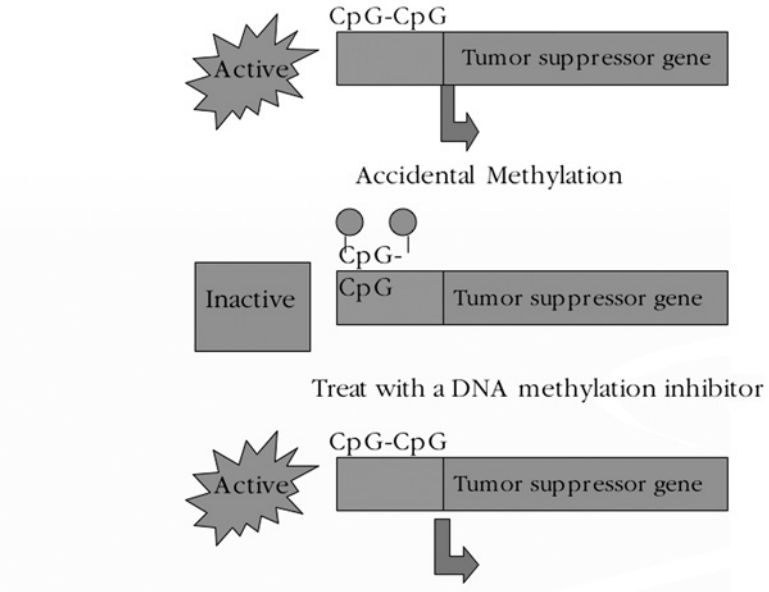


Figure 1. Regulation of transcription by DNA methylation.

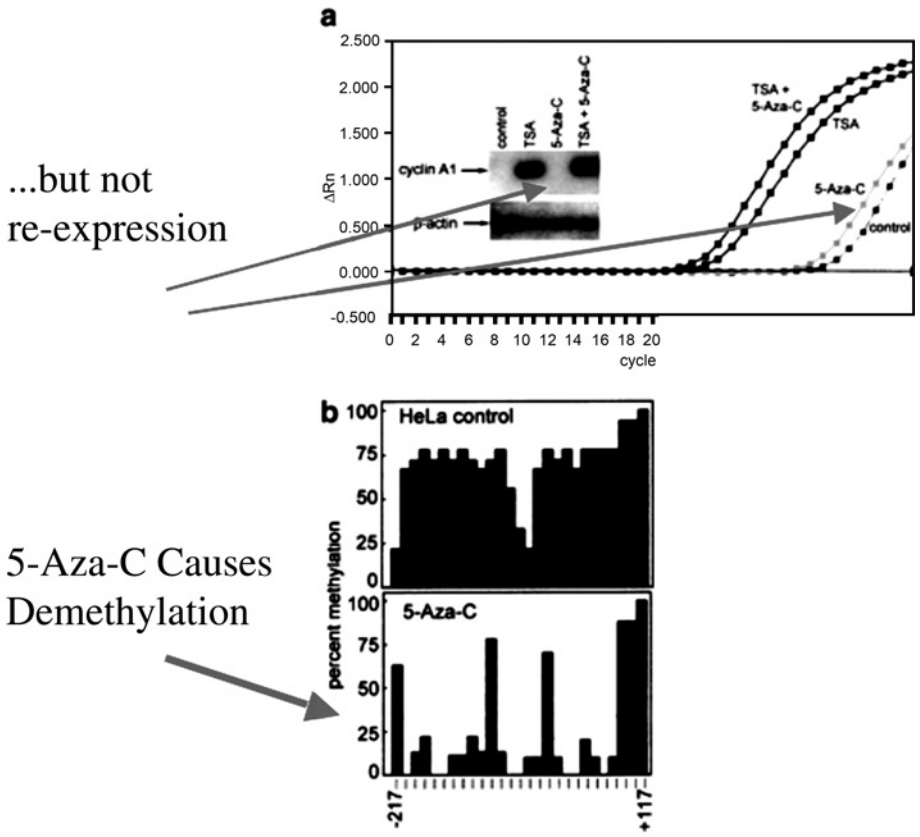


Figure 2. Demethylation does not always result in reexpression of functional tumor suppressor proteins. Reprinted with permission from [3].

mutation or deletion of the first allele, so dysfunctional tumor suppressors can still occur when one allele of a tumor suppressor (in a heterozygous state) has additionally been inactivated by DNA methylation.

Chemical Structure and Function of Methyltransferase Inhibitors

The chemical structures of several DNA methyltransferase inhibitor drugs are shown in Figure 3 [3]. When cells incorporate these DNA methyltransferase inhibitors, the DNA methyltransferase enzyme becomes covalently bound to the genome that has incorporated these drugs. The only difference between azacitidine and decitabine is that azacitidine has an extra hydroxyl group in the 2 prime part of the sugar. When an inhibitor such as azacitidine or decitabine is incorporated into the genome, it stops DNA methyltransferase and forms DNA adducts. These are protein adducts that are potentially lethal to the cells. If the cells are able to degrade these adducts, then they are able to continue to replicate and survive. If cells are unable to degrade these adducts and the adducts form and accumulate, mutational events increase and continue to be replicated in the genome.

Although azacitidine and decitabine differ chemically only by the 2 prime hydroxyl group, azacitidine has been shown to be incorporated into the DNA and the RNA of the genome. This feature is likely an advantage, as indicated by the clinical responses we have seen compared to Decitabine, which is incorporated only into the DNA.

The DNA methyltransferase inhibitors are relatively nonselective in that they inhibit all 3 DNA methyltransferases found in humans, DNA methyltransferase I, IIIA, and IIIB. DNA methyltransferase I is thought to be the predominant DNA methyltransferase because it keeps the maintenance methylation pattern in the cell; IIIA and IIIB are responsible for de novo methylation patterns and are thought to be more important during the fetal period.

Administration of Methyltransferase Inhibitors

All of the DNA methyltransferase inhibitors have several characteristics in common. They are short in half-life, so that that in blood samples collected after administration of the drug, the parent compound is gone after approximately 20 minutes. These drugs are very unstable in solution, so they must be reconstituted prior to administration. Because these drugs have a relatively short half-life and are relatively unstable there is an advantage to administering them frequently at low doses rather than by continuous intravenous infusion. Administration of low doses decreases the number of genomic mutations introduced into the cells and decreases the interval between treatments. Low-dose administration also decreases the cytopenia and fatigue-related symptoms experienced by patients treated with these drugs.

The standard dose of the DNA methyltransferase inhibitors is rather cytotoxic. As a result, a lot of cells die and even the cells that have been completely demethylated are not measurable, so there has been some difficulty in correlating the clinical response to a molecular response. Low doses will allow better measurement and correlation of inhibition of DNA methylation to a clinical response, which will enable determination of the dosing schedules.

The DNA methyltransferase inhibitors have been demonstrated to be very effective in MDS, and at least transient responses have been seen in patients with acute myelogenous leukemia. These drugs are not very effective as single agents in the treatment of solid tumors, however, and must be combined with other agents to make them more effective.

Conclusion

The DNA methyltransferase inhibitors are incorporated into the genome, and cause DNA damage and introduce mutational events. Because they have to be incorporated, the drugs are effective only in replicating

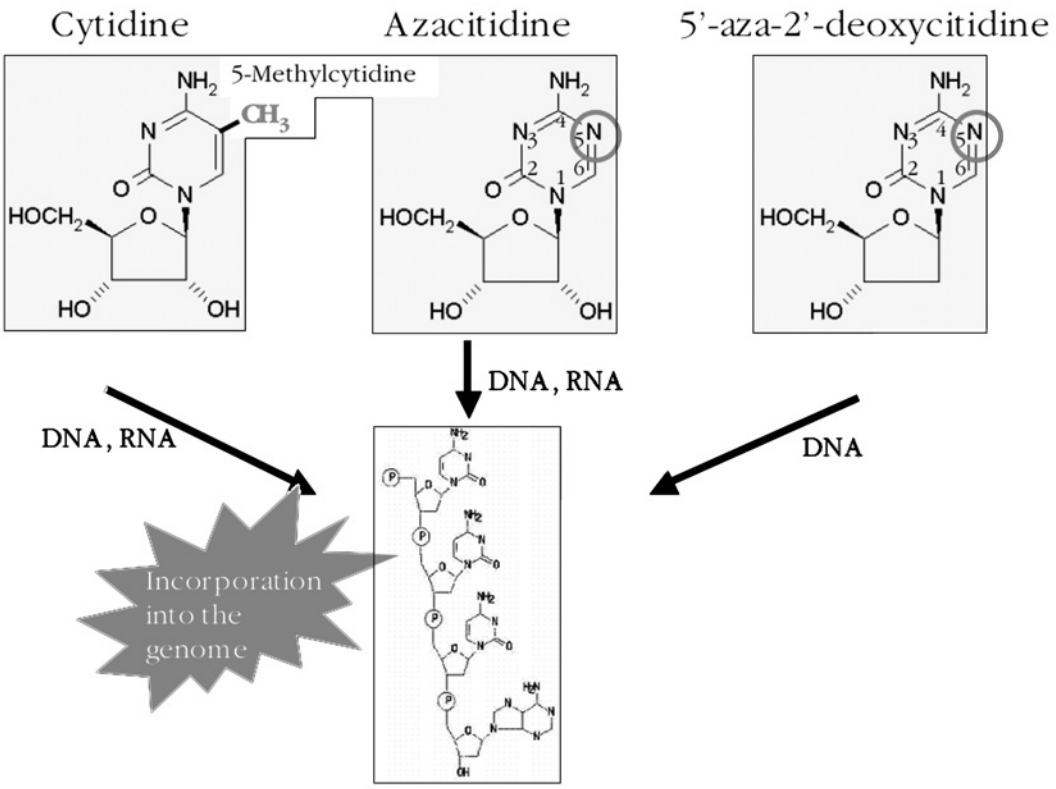


Figure 3. Chemical structure of 3 DNA methyltransferase inhibitors.

cells. Further research will seek to clearly demonstrate that these drugs have been incorporated and that their incorporation corresponds to reexpression of a gene, whether that be a tumor antigen, that we know is normally methylated in the cell or is one of the genes that has been identified as important for the pathogenesis of a disease. And lastly gene expression must be correlated to transcript copies in the mRNA as well as the protein level.

References

1. Guo SX, Taki T, Ohnishi H, et al. Hypermethylation of p16 and p15 genes and RB protein expression in acute leukemia. *Leuk Res*. 2000;24: 39-46.
2. Daskalakis M, Nguyen TT, Nguyen C, et al. Demethylation of a hypermethylated P15/INK4B gene in patients with myelodysplastic syndrome by 5-Aza-2'-deoxycytidine (decitabine) treatment. *Blood*. 2002; 100:2957-2964.
3. Muller C, Readhead C, Diederichs S, et al. Methylation of the cyclin A1 promoter correlates with gene silencing in somatic cell lines, while tissue-specific expression of cyclin A1 is methylation independent. *Mol Cell Biol*. 2000;20:3316-3329.

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.

Kasamon YL, Jones RF, Diehl LF, et al: Outcomes of autologous and allogeneic blood or marrow transplantation for mantle cell lymphoma. Biol Blood Marrow Transplant. 2005;11:39-46.

Mantle cell lymphoma does not usually respond well to standard chemotherapy, with most patients experiencing relapse within 18 months. High-dose systemic therapy plus blood or bone marrow transplantation (BMT) might offer an important alternative, but the survival benefits remain unclear. The authors analyze their center's experience with autologous and allogeneic BMT for mantle cell lymphoma.

A review of the Johns Hopkins BMT Registry identified 58 patients who underwent BMT for new or relapsed mantle cell lymphoma between 1993 and 2003. At the time of BMT, median age was 55 years and 88% of patients had Ann Arbor stage III or IV disease. Bone marrow transplantation was performed in first remission in 64% of patients, after a relapse in 24%, and after failed primary induction therapy in 12%. Autologous BMT was performed in 66% of patients and allogeneic BMT in 33%—1 patient received a syngeneic graft. The preparative regimen generally consisted of cyclophosphamide with total body irradiation or busulfan.

At last follow-up, 12 relapses had occurred, 8 of them among patients receiving autologous BMT. Median event-free survival (EFS) was estimated at 43 months with overall survival of 52 months. On multivariate analysis, factors associated with lower EFS were BMT after first or later relapse, hazard ratio (HR) 2.98; BMT after primary induction failure, HR 5.39; and allogeneic BMT, HR 3.03. Among patients transplanted in first remission, EFS was similar with autologous vs allogeneic BMT—both groups had an estimated 3-year EFS of approximately 70%.

Independent predictors of relapse were primary induction failure and residual bone marrow involvement. Three years after BMT, estimated EFS was 51%, with a 31% probability of relapse and 59% overall survival.

A relatively large experience with autologous or allogeneic BMT for mantle cell lymphoma is reviewed. Outcomes appear best for patients undergoing BMT in first remission—results are similar with autologous or allogeneic marrow. The outcomes are not as good for patients transplanted after relapse or primary induction failure. Further study is needed to determine whether allogeneic BMT may offer advantages through a graft-vs-lymphoma effect.

Zhang S, Guo J, Zhang P, et al: Long-term effects of bone marrow molecular cell transplantation on left ventricular function and remodeling in rats. Life Sci. 2004;74:2853-2864.

Left ventricular remodeling is a major factor affecting patient outcomes after myocardial infarction. Recent studies have suggested that some bone marrow mononuclear cell (BM-MNC) subtypes promote regeneration of blood vessels and necrotic heart muscle. Some effects of peripheral blood mononuclear cell (PB-MNC) transplantation have been reported as well. The effects of BM-MNC transplantation on left ventricular remodeling were assessed in a rat model of acute myocardial infarction.

Myocardial infarction was induced in Lewis rats by ligation of the left anterior descending coronary artery. The infarct and peri-infarct area was then injected with PB-MNCs or BM-MNCs, 5×10^6 cells in three sites; or with saline solution. Echocardiography was performed before infarction and at 1 day and 2 months afterward to assess cardiac structure and function. Histologic and immunohistochemical studies were performed to assess collagen content, blood vessel numbers, and vasculogenesis.

On the day after infarction, all three groups of rats had typical findings of acute heart failure and early left ventricular remodeling. At 2 months' follow-up, rats receiving BM-MNCs had significantly improved cardiac systolic function, along with recovery of diastolic function. Left ventricular fractional shortening was 57% greater than in rats receiving saline solution and 26% greater than in animals receiving PB-MNCs. Bone marrow cell transplantation was associated with near-complete recovery of indicators of left ventricular remodeling.

Animals receiving BM-MNCs also had reduced collagen density and increased evidence of angiogenesis. The transplanted bone marrow cells showed evidence of a vascular endothelial cell-specific cytoplasmic protein, factor VIII.

After experimentally induced myocardial infarction in rats, transplantation with BM-MNCs yields persistent improvement in left ventricular remodeling and function, compared with PB-MNC transplantation or saline injection. Bone marrow transplantation seems to have the greatest impact on diastolic function. The mechanism of these benefits may involve reduction of collagen accumulation and increased neovascularization.

Scott BL, Storer B, Loken MR, et al: Pretransplantation induction chemotherapy and posttransplantation relapse in patients with advanced myelodysplastic syndrome. Biol Blood Marrow Transplant. 2005;11:65-73.

The only potentially curative treatment option for myelodysplastic syndrome (MDS) is hematopoietic cell transplantation, which offers excellent outcomes for patients with certain favorable characteristics. However, relapse rates are high for those with various forms of advanced MDS or with transformation to acute myeloid leukemia with multilineage dysplasia (tAML). Induction chemotherapy (IC) has high toxicity, but may be given to reduce the risk of relapse. The effects of pretransplant IC on relapse rate and survival in patients with advanced MDS were analyzed.

The retrospective study included 125 patients undergoing hematopoietic cell transplantation for advanced MDS or tAML. The transplants were performed between 1992 and 2002; all patients received hematopoietic cells from HLA-identical related or unrelated donors. Induction chemotherapy was given before transplantation in 33 patients: 3 had refractory anemia with excess blasts (RAEB), 6 had RAEB in transformation (RAEB-T), and 24 had tAML. The remaining 92 patients—62 with RAEB, 22 with RAEB-T, and 8 with tAML—did not receive IC. About 60% of patients underwent conditioning with oral busulfan 16 mg/kg, adjusted to a steady-state plasma concentration of 800 to 900 ng/mL, plus cyclophosphamide 2×60 mg/kg. The remaining 40% received busulfan 7 mg/kg, with not dose adjustment, plus total body irradiation 6×200 cGy over 3 days. Relapse and survival outcomes were compared for patients who did and did not undergo IC.

Of the 33 patients treated with IC, 18 had complete remission while 11 had no response. Five patients experienced relapse before transplantation was performed. There were no significant differences in the outcomes of hematopoietic cell transplantation between patients who did and did not undergo IC. This was so on analysis of patients with RAEB, RAEB-T, or tAML, as well as for those receiving the differing conditioning regimens. The 3-year cumulative relapse rate was 53% for patients who received IC and 31% for those who did not. Relapse-free survival was similar as well. Regardless of whether they received IC, patients with high pretransplant flow cytometric scores were more likely to experience a relapse.

For patients with advanced MDS or tAML, this retrospective study finds no significant benefit of performing IC before hematopoietic cell transplantation. The posttransplant relapse rates are significantly affected by the response to IC and by the pretransplant flow cytometry score. Randomized trials will be needed to determine the true benefits of IC in this group of patients.

Mullighan C, Heatley S, Doherty K, et al: Non-HLA immunogenetic polymorphisms and the risk of complications after allogeneic hemopoietic stem-cell transplantation. Transplantation. 2004;77(4):587-596.

Even with optimal human leukocyte antigen (HLA) matching, recipients of allogeneic hematopoietic stem cell transplants (SCT) are at risk of graft-vs-host disease (GVHD). Recent studies suggest that the risk and severity of GVHD are affected by non-HLA immunogenetic polymorphisms. The few studies of this issue to date have had important limitations, including few small sample sizes and lack of donor typing. Various non-HLA polymorphisms were evaluated for their relationship to the outcomes of allogeneic SCT.

The study was based on 160 related donor-recipient pairs undergoing myeloablative hematopoietic SCT at three Australian centers during the 1990s. Genotyping studies were performed to evaluate a total of 22 polymorphisms of 11 immunoregulatory genes, including genes for cytokines, apoptosis mediators, and host defense molecules. Polymorphisms potentially related to GVHD and other outcomes were confirmed in two independent cohorts of SCT patients.

An uncommon intronic polymorphism of the tumor necrosis factor gene, TNF 488A, was significantly associated with the occurrence and severity of acute GVHD. All recipients with this allele developed acute GVHD, compared to 69.5% of those without TNF 488A, odds ratio (OR) 16.9. The TNF 488A polymorphism was also associated with grade II to IV acute GVHD, OR 3.3; with chronic GVHD, OR 3.4; and with early death after transplantation, OR 3.4.

The risk of acute GVHD was also independently related to the presence of the *Fas* -670G polymorphism in recipients, OR 3.1; and to the presence of the interleukin (IL)-6 -174 G polymorphism in donors, OR 4.4. Risk of chronic GVHD was affected by the presence in recipients of the IL-10 ATA promoter haplotype, OR 3.9; and by the *Fas* -670 genotype. Recipients with the IL-1β +3953T polymorphism were at higher risk of hepatic acute GVHD, while those with *Fas* -670G were more likely to develop major infectious complications.

Several different non-HLA polymorphisms for cytokines and apoptosis mediators may influence the outcomes of allogeneic SCT. The intronic TNF 488A allele is a strong predictor of acute and chronic GVHD, while the *Fas* genotype affects acute GVHD risk. In the future, non-HLA genotyping may be useful in identifying SCT recipients at the highest risk of GVHD and other complications, and in developing new therapeutic targets.

Staba SL, Escolar ML, Poe M, et al: Cord-blood transplants from unrelated donors in patients with Hurler's syndrome. N Engl J Med. 2004;350:1960-1969.

Hurler's syndrome, or severe mucopolysaccharidosis type I, is an autosomal recessive disorder that leads to progressive CNS deterioration, cardiac and skeletal abnormalities, and early death. Bone marrow transplantation is a life-prolonging treatment if given before age 2; however, most children with Hurler's syndrome have no matched donor. An experience with unrelated cord blood transplantation for children with Hurler's syndrome is reported.

From 1995 to 2002, the authors performed unrelated cord blood transplantation in 20 consecutively referred children with Hurler's syndrome who lacked an HLA-matched related bone marrow donor. All cord blood donors had normal α-L-iduronidase activity, mean cell count 10.53×10^7/kg. Donors and patients were mismatched for up to three of six HLA markers. Pretransplant conditioning included busulfan, cyclophosphamide, and antithymocyte globulin; cyclosporine and methylprednisolone were given as prophylaxis against graft-vs-host disease (GVHD).

Median time to neutrophil engraftment after cord blood transplantation was 24 days. Five of the twenty recipients developed grade II or III acute GVHD, but none developed extensive chronic GVHD. At a median 950 days' follow-up, 17 patients were alive with complete donor chimerism and normal α-L-iduronidase activity, for an event-free survival rate of 85%. Most children had normal growth, none had significant cardiac dysfunction, and all had stable or improved neurocognitive function.

For children with Hurler's syndrome who lack a matched, related donor, unrelated donor cord blood provides an alternative source of hematopoietic stem cells for transplantation. These transplants lead to sustained engraftment without pretransplant total body irradiation, and low rates of GVHD. Follow-up shows improved neurocognitive function and reduced somatic features of this otherwise progressive and fatal disease.

Jacobsohn DA, Schechter T, Seshadri R, et al: Eosinophilia correlates with the presence or development of chronic graft-versus-host disease in children. Transplantation. 2004;77(7):1096-1100.

Information on risk factors for chronic graft-vs-host disease (cGVHD) in children—especially severe or extensive cGVHD—might have important clinical implications. Recent reports indicate that the development of cGVHD may be a Th2-mediated process, suggesting a possible association with eosinophilia. Eosinophilia was evaluated as a risk factor for cGHVD in children receiving hematopoietic stem cell transplants (SCTs).

The retrospective study included 53 patients undergoing SCT at a children's hospital from 1999 to 2002. All patients received total body irradiation and cyclophosphamide, and showed 100% donor chimerism after 100 days. Eosinophilia, defined as an absolute eosinophil count (AEC) of greater than 500×10^6/L anytime after the first 100 days, occurred in 10 of the children, a rate of 19%.

The children with eosinophilia had a mean maximal AEC of $2,477 \times 10^6$, which developed a median of 194 days after SCT. Of the 10 children with eosinophilia, 8 either had cGVHD at the time of eosinophilia or developed it later on. In univariate models, patients with current or previous eosinophilia had a 24.7 odds ratio for cGVHD and a 5.7 odds ratio for extensive cGVHD.

In pediatric SCT recipients, an AEC over 500×10^6 occurring more than 100 days after transplantation is associated with the development of cGVHD. Added to other risk factors such as previous grade II to IV acute GVHD, HLA mismatch, and unrelated donor transplant, eosinophilia may be an important warning sign of cGVHD. The authors now begin treatment for SCT recipients with persistent eosinophilia, even before signs and symptoms of cGVHD develop.

Novel Therapies in the Treatment of Myelodysplastic Syndromes

CME Assessment Test

1. **Which of the following is a NOT true of myelodysplastic syndromes?**
   A. They occur most commonly in elderly patients.
   B. They are a homogeneous group of disorders.
   C. Stem-cell based therapy is currently the only potentially curative treatment.
   D. All of the above.

2. **Which of the following is true regarding MDS diagnosis and classification?**
   A. MDS has a unique clinical presentation and is easily distinguished from other disorders.
   B. Hypercellular marrow, peripheral cytopenia, ineffective hematopoeisis, and cellular functional abnormalities indicate that a patient does not have MDS.
   C. Characterization of disease stage and severity using the FAB/WHO and IPSS classification systems are important for treatment planning.
   D. All of the above.

3. **Which of the following is true regarding MDS classification systems?**
   A. WHO modifications are based on the recognition that there are subsets of patients whose disease does not fit FAB criteria.
   B. The FAB scoring system does not always correlate with disease natural history in MDS.
   C. In terms of survival, the IPSS score correlates very well with MDS natural history.
   D. All of the above.

4. **Which of the following is true regarding transplantation for treatment of MDS?**
   A. Like all treatment options, transplantation is not potentially curative.
   B. Most MDS patients are good candidates for transplantation.
   C. Less aggressive pretransplantation disease control increases the risk of relapse later.
   D. All of the above.

5. **Which of the following is true of best supportive care for MDS?**
   A. The methyltransferase inhibitor azacitidine may be considered as a new standard of best supportive care.
   B. MDS patients rarely require blood transfusions.
   C. Thrombopoietic agents have significantly reduced transfusion needs in MDS patients.
   D. All of the above.

6. **Which of the following is true of standard chemotherapy for MDS?**
   A. Studies have shown that MDS patients do not benefit from low-dose cytarabine or other forms of standard chemotherapy.
   B. AML-type induction chemotherapy may be beneficial for younger patients with normal cytogenetics without a prior history of abnormal blood count.
   C. Chemotherapy may have a role in patients who will undergo autologous stem cell transplantation or who may be candidates for reduced-intensity transplantation.
   D. All of the above.

7. **Which of the following is true of new treatment agents for MDS?**
   A. Thalidomide is well tolerated by all MDS patients.
   B. CC5013 (lenalidomide), an immunomodulatory drug that is chemically similar to thalidomide, may actually improve cytogenetic abnormalities in MDS patients.
   C. Improved survival of MDS patients treated with azacitidine was limited to those with FAB low-risk disease.
   D. All of the above.

8. **Which is the following is true regarding DNA methylation in normal cells?**
   A. Normal cells have a higher level of global methylation than cancer cells.
   B. DNA methylation plays an important role in X-chromosome inactivation in normal fetal development.
   C. DNA methylation silences genes in normal cells.
   D. All of the above.

9. **Which of the following is true regarding the pharmacology of the methyltransferase inhibitors?**
   A. DNA methyltransferase inhibitors can reverse methylation only in replicating cells.
   B. The only difference between azacitidine and decitabine is that azacitidine has an extra hydroxyl group in the 2 prime part of the sugar.
   C. Azacitidine has been shown to be incorporated into the DNA and the RNA of the genome, whereas decitabine is incorporated only into the DNA.
   D. All of the above.

10. **Which of the following is true regarding administration of methyltransferase inhibitors?**
    A. These drugs are very unstable in solution, so they must be reconstituted prior to administration.
    B. The best way to administer these drugs is by intravenous infusion.
    C. Low-dose administration does not decrease the cytopenia and fatigue-related symptoms experienced by patients treated with these drugs
    D. All of the above.

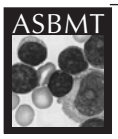
CME Assessment Test Answer Sheet

Release Date: May 31, 2005 **Last Review Date: May 31, 2005** **Expiration Date: May 31, 2006**

Instructions

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

1.	A	B	C	D	**5.**	A	B	C	D	**8.**	A	B	C	D
2.	A	B	C	D	**6.**	A	B	C	D	**9.**	A	B	C	D
3.	A	B	C	D	**7.**	A	B	C	D	**10.**	A	B	C	D
4.	A	B	C	D										



CME Evaluation Form

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

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

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

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

I have read these articles on novel therapies in the treatment of myelodysplastic syndromes, published in *Blood and Marrow Transplantation Reviews*, and have answered the CME test questions and completed the Evaluation Form for this activity.

Signature Date

Last Name First Name MI Degree

Specialty Affiliation

Address

City State Postal Code

Phone Fax E-mail

ASBMT™
American Society for Blood and Marrow Transplantation

Non-Profit Organization
U.S. Postage
PAID
Charlottesville, Virginia
Permit No. 232