



Michele Sassi. *Fall in the Berkshires*. Photograph. Berkshires, Massachusetts.

*Supportive care alone for MDS
appears inferior to newer
treatment approaches using
methyltransferase inhibitors.*

The Role of Methyltransferase Inhibitors in the Management of the Myelodysplastic Syndromes

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The novel class of therapeutics called methyltransferase inhibitors (MTIs) offers a significant addition to the treatment armamentarium for clinicians who care for patients with the myelodysplastic syndromes (MDS). Two MTIs — azacitidine (Vidaza[®], Pharmion Inc; Boulder, Colo), approved by the Food and Drug Administration (FDA) in June 2004, and decitabine (Dacogen[®], SuperGen Inc; Dublin, Calif), in development — have been tested in patients with MDS.^{1,2} These agents produce broad biologic effects, including the induction of cell differentiation in vitro, mediated in part by inhibiting the enzyme responsible for methylation of DNA (DNA methyltransferase) and thus leading to DNA hypomethylation.^{1,2} The recent FDA approval for azacitidine provided an indication for treatment of MDS patients classified according to French-American-British (FAB) criteria and encompassing all five subtypes. With the availability of azacitidine, clinicians need to understand the pharmacological mechanisms of action

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of these agents and how they influence the biology of the myelodysplastic disease process. Furthermore, oncologists need to be familiar with the clinical trial experience with these agents in order to apply them effectively and safely in the management of MDS.

Pharmacologic Basis of Methyltransferase Inhibition

Azacitidine was originally synthesized by Czechoslovakian scientists in the 1960s and examined in clinical trials in the United States in the 1970s.³ Developed as a cytotoxic agent, azacitidine in leukemia was first discussed by Massimo and Comelli⁴ in 1975. Although active as a single agent in relapsed and refractory acute myeloid leukemia (AML), it never advanced through the FDA review process as a leukemia therapy. Effects of the MTIs on cell differentiation resulting in an alteration of the malignant phenotype in vitro led to testing in patients with hemoglobinopathies, specifically, beta thalassemia and sickle cell anemia.^{5,6} These studies demonstrated an increased production of fetal hemoglobin after treatment with azacitidine and an association with hypomethylation of the γ globulin chain gene, leading to amelioration of symptoms.⁷⁻¹⁰ On this basis, studies with azacitidine were undertaken in patients with MDS.

Interest in azacitidine in MDS was predicated on the basis of its effects on DNA methylation and cell differentiation. Jones and Taylor^{11,12} found that azacitidine could

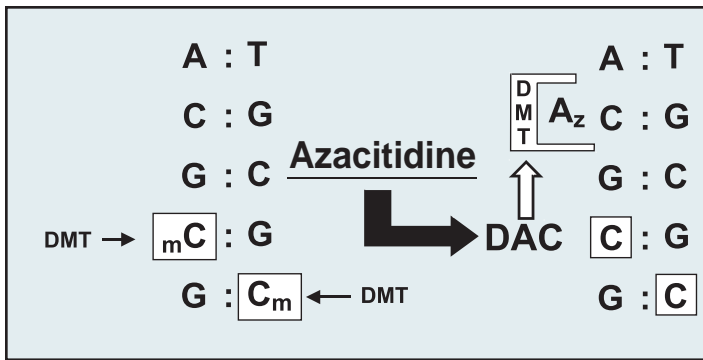


Fig 1. — Azacitidine is incorporated into DNA in lieu of cytosine residue, inactivates DNA methyltransferase (DMT), leads to formation of newly synthesized DNA with unmethylated cytosine residues, and results in hypomethylation and transcription of previously quiescent genes. From Silverman LR. Targeting hypomethylation of DNA to achieve cellular differentiation in myelodysplastic syndromes (MDS). *Oncologist*. 2001;6(suppl 5):8-14. Permission from *The Oncologist*, AlphaMed Press.

induce differentiation of erythroleukemic cells in vitro and that its incorporation into DNA resulted in a time-dependent inhibition of DNA methyltransferase activity. Azacitidine is incorporated into DNA as a substituted cytosine residue that then irreversibly binds to and inhibits DNA methyltransferase (Fig 1). This interaction results initially in hemi-methylated DNA that, after another cell cycle and round of DNA synthesis, becomes fully unmethylated. This unmethylated DNA can lead to subsequent transcription of previously quiescent genes.¹³ This sequence suggests a biochemical model and explanation for the action of the MTIs, its effect on gene transcription, and the clinical potential for epigenetic modulation.

The degree of methylation of the CpG islands plays a role in the control of gene transcription. Fully methylated sites are associated with suppression of gene expression, while hypomethylated or unmethylated CpG islands are associated with active transcription. Areas in the genome that become hypermethylated, relative to their natural state, lead to silencing of potentially important genes.^{14,15} This may cause inhibition or silencing of tumor suppressor genes and other genes critical in regulation of the cell

cycle and cell differentiation.¹⁶ In MDS, this mechanism may interrupt the regulation of normal hematopoiesis.¹⁴⁻¹⁸

In addition to acting as DNA MTIs, azacitidine and decitabine also may inhibit DNA histone acetylation.¹⁹ Histone acetylation plays a critical role in chromatin structure, which when modified can either facilitate or hinder access to genes. This interaction affects transcription and represents another regulatory mechanism in silencing genes.²⁰

Azacitidine Trials

Based on the observations of the effect of azacitidine in cell differentiation and gene transcription, the Cancer and Leukemia Group B (CALGB) undertook a phase II study to explore efficacy in patients with advanced or higher risk forms of MDS.²¹ Azacitidine was administered at a daily dose of 75 mg/m² as a continuous intravenous infusion for 7 consecutive days repeated in 28-day cycles. Patients with MDS classified according to FAB criteria — refractory anemia with excess blasts (RAEB) or refractory anemia with excess blasts in transformation (RAEB-T) — were treated for 4 months. Responding patients continued on treatment with monthly cycles. Treatment was stopped in nonresponders and in those with stable disease or progression.

Azacitidine produced a response rate of 49% and was well tolerated, suggesting that it might produce a significant effect in MDS.^{21,22}

Because administration required hospitalization for 7 days, a subsequent trial tested azacitidine administered as a single subcutaneous bolus injection at 75 mg/m² daily for 7 consecutive days. Cycles were repeated every 28 days for a minimum of 4 months in an ambulatory setting.²² Patients were enrolled if they had RAEB, RAEB-T, or chronic myelomonocytic leukemia (CMML), according to FAB criteria. This trial yielded an overall response rate of 53%, which was comparable in both safety and efficacy to the previous intravenous formulation study. Response to treat-

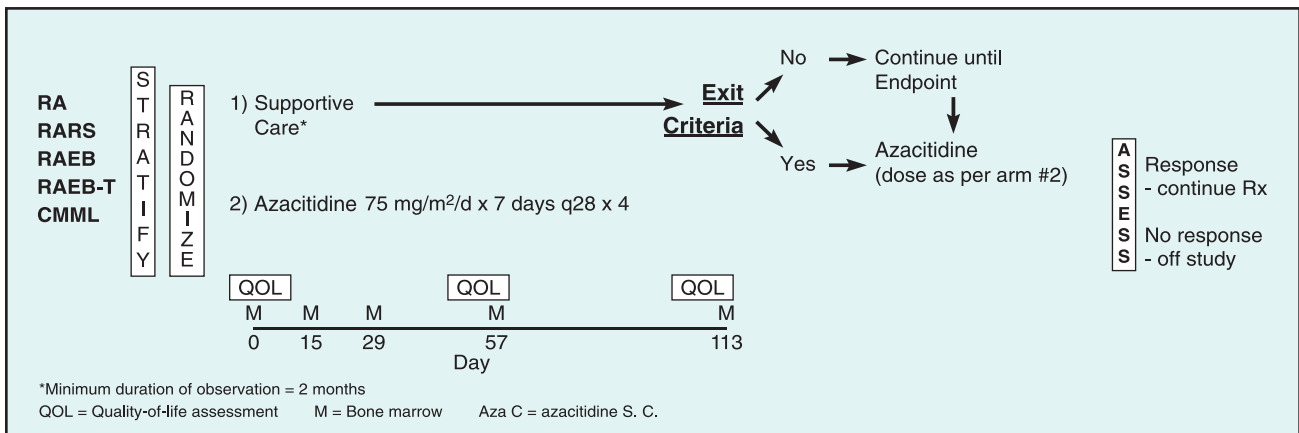


Fig 2. — CALGB 9221 trial design. MDS patients were stratified and randomized to best supportive care or azacitidine groups. If patients in the best supportive care arm progressed, they were allowed to cross over into the treatment group. From Silverman LR. Targeting hypomethylation of DNA to achieve cellular differentiation in myelodysplastic syndromes (MDS). *Oncologist*. 2001;6(suppl 5):8-14. Permission from *The Oncologist*, AlphaMed Press.

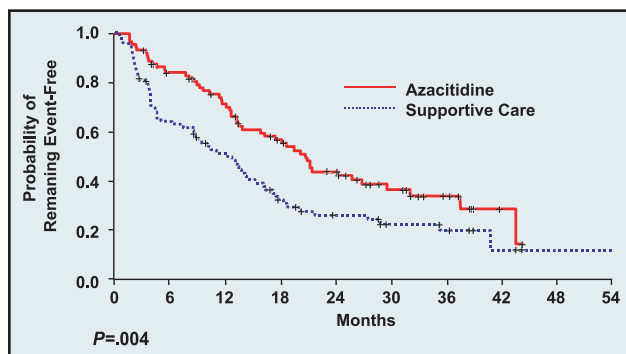


Fig 3. — Time to AML or death in patients with MDS. From Silverman LR, Demakos EP, Peterson BL, et al. Randomized controlled trial of azacitidine in patients with the myelodysplastic syndrome: a study of the Cancer and Leukemia Group B. *J Clin Oncol.* 2002;20:2429-2440. Reprinted with permission of the American Society of Clinical Oncology.

ment with azacitidine was slow, with a median time to response of 91 and 95 days. Although treatment was associated with exacerbation of preexisting cytopenias in some patients, bone marrow hypoplasia occurred in only 10% of those treated.²²

Based on the promising results seen within the phase II investigations, the CALGB conducted a randomized phase III study (CALGB 9221) to compare azacitidine to best supportive care in 191 patients with a diagnosis of MDS according to FAB criteria.²³ Patients with all FAB subtypes were included. Those with refractory anemia (RA) and refractory anemia with ringed sideroblasts (RARS) had to be symptomatic, either requiring red cell transfusions, having a platelet count of $\leq 50,000/\mu\text{L}$, or having an absolute neutrophil count $\leq 1,000/\mu\text{L}$ with an infection. Azacitidine was administered as a single daily subcutaneous bolus injection for 7 consecutive days every 28 days at the same dosage as in the previous two studies (Fig 2).²³ Patients were to remain in the supportive care group for a minimum of 4 months unless they demonstrated evidence of progression to AML, succumbed to disease, or had a platelet count of $\leq 20,000/\mu\text{L}$ following week 8. After 4 months, patients could cross over to receive treatment

Table 1. — Response to Treatment With Best Supportive Care vs Azacitidine in the CALGB Trial in MDS

Response	Supportive Care (n = 92)	Azacitidine (n = 99)	Crossover (n = 49)
Complete response	0 (0%)	7 (7%) *	5 (10%)
Partial response	0 (0%)	16 (16%) **	2 (4%)
Improvement	5 (5%)	37 (37%) **	16 (33%)
Total	5 (5%)	60 (60%) **	23 (47%)

* $P < .01$.
** $P < .0001$.

From 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. Reprinted with permission from the American Society of Clinical Oncology.

with azacitidine if they had defined worsening of the MDS or transformed to AML. Those individuals within the azacitidine group exited that group if they did not achieve a response or if they progressed.²³

There were no significant differences between groups with respect to diagnosis or other demographics. Responses were categorized according to similar criteria utilized in the two previous studies and included complete response, partial response, or improvement. Lineage responses were defined as a reduction by 50% or greater in the deficit from normal in a specific lineage, either platelet, red cell, or white cell. Patients who responded in all three cell lines were considered to have a trilineage response. Patients who had a partial or complete response had to have an elimination of all transfusion requirements and reductions in the blast count in the bone marrow.²³ Responses were seen in all subtypes, with a response rate of approximately 60% in patients with RAEB and RAEB-T. Comparable responses were seen in patients with RA and RARS, representing the first experience of azacitidine in these “low risk” FAB subgroups. The median duration of response for all patients was 14 months (Table 1). Time to AML or death (Fig 3) was significantly longer for the azacitidine group than for the supportive care group (median time of 21 months and 12 months, respectively).²³ The incidence of transformation to AML also significantly decreased in the azacitidine group.

A landmark analysis of patients alive at 12 months demonstrated that those who progressed to AML had only a median additional survival of 3 months compared with 18 months for patients who had not progressed to AML by the 12-month landmark ($P < .001$). The overall survival favored patients in the azacitidine group, with a median duration of 20 months compared with 14 months in the supportive care group. The result was not significantly different ($P = .10$), possibly confounded by the crossover

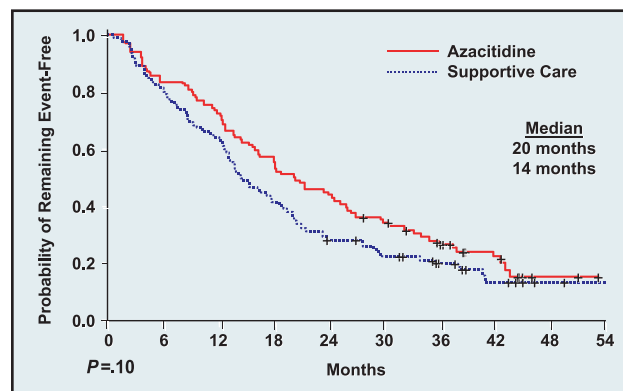


Fig 4. — CALGB study comparing duration of response in 191 patients with MDS. Median survival was 20 months for patients randomized to azacitidine vs 14 months for those receiving supportive care. From Kornblith AB, Herndon JE, Silverman LR, et al. Impact of azacitidine on the quality of life of patients with myelodysplastic syndrome treated in a randomized phase III trial: a Cancer and Leukemia Group B study. *J Clin Oncol.* 2002;20:2441-2452. Reprinted with permission of the American Society of Clinical Oncology.

design (Fig 4). A landmark analysis of patients alive at 6 months demonstrated a significant survival advantage for patients treated initially with azacitidine compared with those not treated or who received only 6 months of supportive care ($P=.03$).²³

An assessment of quality of life was conducted as part of the study and reported by Kornblith et al.²⁴ The European Organization for Research and Treatment of Cancer (EORTC) dyspnea and fatigue scale and the Mental Health Inventory (MHI) psychological well-being subscale assessment subscore were used. The results demonstrated that fatigue and dyspnea worsened for patients in the supportive care group and improved for patients in the azacitidine group. These symptoms did not abate in the patients in the supportive care group despite continued administration of red cell transfusions. Similarly, the MHI subscale demonstrated stable or worsening psychological well-being for those in supportive care compared with stable to improving MHI for patients treated with azacitidine. Quality-of-life assessments continued for patients in the best supportive care group who crossed over to receive treatment with azacitidine. Figure 5 depicts the cumulative results. Dyspnea, fatigue, and physical functioning worsened while receiving best supportive care, but all of these parameters improved after crossover.²⁴

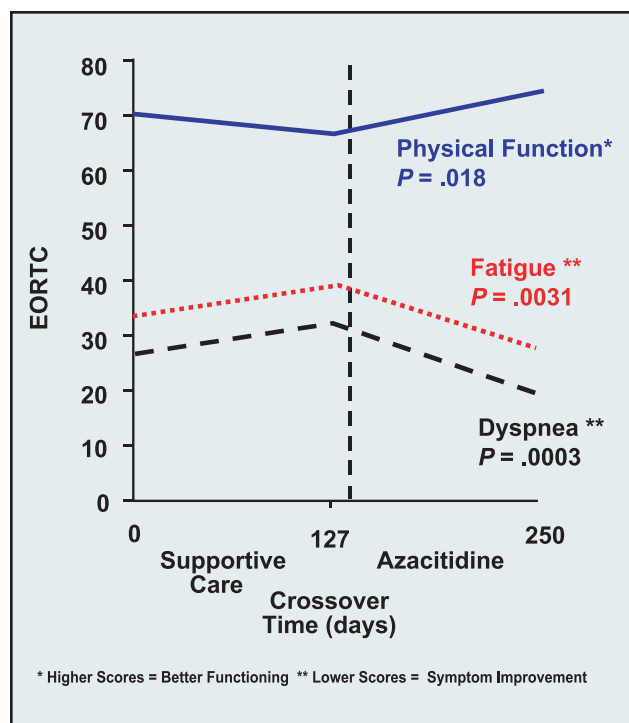


Fig 5. — EORTC comparison of quality-of-life measures before and after crossover from supportive care to azacitidine therapy. Symptoms of fatigue and dyspnea decreased, and physical functioning improved in 30 patients who crossed over to azacitidine after approximately 4 months on supportive care. From Kornblith AB, Herndon JE, Silverman LR, et al. Impact of azacytidine on the quality of life of patients with myelodysplastic syndrome treated in a randomized phase III trial: a Cancer and Leukemia Group B study. *J Clin Oncol*. 2002;20:2441-2452. Reprinted with permission from the American Society of Clinical Oncology.

Decitabine Trials

Decitabine is another hypomethylating agent in development that is being evaluated in patients with MDS.^{25,26} The effect of low-dose decitabine has been reported in 66 patients with high-risk MDS. Thirteen patients achieved complete remission, and 9 patients had trilineage responses.²⁷ Cytopenias delayed subsequent treatment in a number of patients, particularly in the first cycle. The treatment-related mortality rate was 7% compared with only 1% to 2% for studies with azacitidine. The median duration of response with decitabine was 31 weeks compared with the 14 months seen in the phase III study with azacitidine.^{23,27}

Decitabine treatment produces relative hypomethylation of the *p15* gene, which has been implicated in transformation from MDS to AML when hypermethylated. However, there is no correlation between response to decitabine and changes in either baseline methylation status or changes in subsequent methylation states.^{28,29} Further analysis is required to determine the effect of the hypomethylating agents with respect to reversal of epigenetic gene silencing and in MDS. The lack of a specific pathophysiological gene target makes this endeavor difficult.

Conclusions

While reasonable for some patients, best supportive care may be inferior to the newer approaches. MTIs, including azacitidine and decitabine, offer promise for the treatment of MDS. To date, azacitidine is the only agent shown to be superior to best supportive care in a randomized trial. With the availability of these new agents, it will be important for clinicians to learn how use them most effectively within the overall treatment algorithm of MDS. It appears important that patients with MDS receive full courses of therapy (7 days per cycle and a minimum of 4 cycles) to ensure full benefit from the hypomethylation effects of these agents. These and other new agents on the horizon will ultimately change the natural history of MDS and create a new management paradigm for this condition.

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