Original Article

Continued Azacitidine Therapy Beyond Time of First Response Improves Quality of Response in Patients With Higher-Risk Myelodysplastic Syndromes

Lewis R. Silverman, MD[1]; Pierre Fenaux, MD[2]; Ghulam J. Mufti, MD[3]; Valeria Santini, MD[4]; Eva Hellström-Lindberg, MD[5]; Norbert Gattermann, MD[6]; Guillermo Sanz, MD[7]; Alan F. List, MD[8]; Steven D. Gore, MD[9]; and John F. Seymour, MBBS[10]

BACKGROUND: In the AZA-001 trial, azacitidine (75 mg/m^2/d subcutaneously for Days 1-7 of every 28-day cycle) demonstrated improved survival compared with conventional care regimens in patients with International Prognostic Scoring System-defined intermediate-2- or high-risk myelodysplastic syndrome and World Health Organization-defined acute myeloid leukemia with 20% to 30% bone marrow blasts. **METHODS:** This secondary analysis of the AZA-001 phase 3 study evaluated the time to first response and the potential benefit of continued azacitidine treatment beyond first response in responders. **RESULTS:** Overall, 91 of 179 patients achieved a response to azacitidine; responding patients received a median of 14 treatment cycles (range, 2-30). Median time to first response was 2 cycles (range, 1-16). Although 91% of first responses occurred by 6 cycles, continued azacitidine improved response category in 48% of patients. Best response was achieved by 92% of responders by 12 cycles. Median time from first response to best response was 3.5 cycles (95% confidence interval [CI], 3.0-6.0) in 30 patients who ultimately achieved a complete response, and 3.0 cycles (95% CI, 1.0-3.0) in 21 patients who achieved a partial response. **CONCLUSIONS:** Continued azacitidine therapy in responders was associated with a quantitative increase in response to a higher response category in 48% of patients, and therefore may enhance clinical benefit in patients with higher-risk MDS. ***Cancer* 2011;117:2697–702.** © *2011 American Cancer Society.*

KEYWORDS: azacitidine, myelodysplastic syndrome, quality of response, higher-risk disease, treatment duration.

The prognosis of patients with International Prognostic Scoring System-defined intermediate-2- or high-risk myelodysplastic syndrome (MDS) is poor, with a median survival of 1.2 and 0.4 years, respectively.[1] Most deaths are attributable to infection, hemorrhage, complications related to anemia or transfusions, or transformation to acute myelogenous leukemia (AML). Consequently, the primary goals of treatment for patients with International Prognostic Scoring System-defined intermediate-2- and high-risk disease are to improve bone marrow function, suppress AML transformation, and prolong survival. Allogeneic stem cell transplantation remains the only potentially curative treatment for MDS. However, the vast majority of patients with MDS are ineligible for transplantation because of advanced age, comorbidities, or the lack of a suitable donor.[2]

The international phase 3 trial AZA-001 established that the cytidine nucleoside analog azacitidine significantly prolonged median overall survival by >9 months and significantly delayed median time to AML transformation by >6 months compared with conventional care regimens.[3] Azacitidine was also associated with significant improvements in other clinically relevant outcomes, including reductions in transfusion need, hospitalization and intravenous antimicrobial

Corresponding author: Lewis R. Silverman, MD, Division of Hematology/Oncology, Tisch Cancer Institute, Mount Sinai School of Medicine, One Gustave L. Levy Place, Box 1129, New York, NY 10029; Fax: (212) 348-9233; lewis.silverman@mssm.edu

[1]Department of Medicine - Oncology, Mount Sinai School of Medicine, New York, New York; [2]Hematology Service, Avicenne Hospital, University of Paris XIII, Bobigny, France; [3]Department of Haematological Medicine, King's College Hospital, London, United Kingdom; [4]Hematology Unit, Careggi University Hospital, Florence, Italy; [5]Department of Hematology, Karolinska University Hospital (Huddinge), Stockholm, Sweden; [6]Department of Hematology, Oncology & Clinical Immunology, Heinrich-Heine University Düsseldorf, Düsseldorf, Germany; [7]Hematology Department, La Fe University Hospital, Valencia, Spain; [8]Department of Hematology, H. Lee Moffitt Cancer Center and Research Institute, Tampa, Florida; [9]Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, Maryland; [10]Department of Haematology, Peter MacCallum Cancer Centre, Melbourne, Australia

DOI: 10.1002/cncr.25774, **Received:** June 8, 2010; **Revised:** October 4, 2010; **Accepted:** October 11, 2010, **Published online** January 10, 2011 in Wiley Online Library (wileyonlinelibrary.com)

use,[3] and improvements in quality of life.[4] In this trial, treatment with azacitidine was continued regardless of response until disease progression or unacceptable toxicity; patients assigned to azacitidine were treated for a median of 9 cycles.[3]

Azacitidine is incorporated into both DNA and RNA. DNA incorporation decreases DNA hypermethylation, allowing the re-expression of previously silenced genes that may include tumor suppressor genes.[5] The incorporation of azacitidine into DNA is cell cycle S-phase dependent and requires exposure during multiple cycles of DNA replication,[6] with hypomethylation occurring gradually; therefore, multiple or prolonged exposure to the drug is required for maximal demethylation. Furthermore, when azacitidine is discontinued, aberrant promoter methylation and gene silencing return. Results from previous clinical studies suggest that continued azacitidine therapy may increase patient benefit.[7-10] Therefore, we conducted a secondary analysis of data from the AZA-001 patients who achieved a response to azacitidine therapy, to determine the time to first response, and whether continued treatment was associated with further improvement in response category.

MATERIALS AND METHODS

This analysis included all patients who responded to azacitidine during the phase 3, international, multicenter, randomized, controlled, parallel-group, open-label AZA-001 trial; this has been described in full previously.[3] The original phase 3 trial (Clinicaltrials.gov: NCT00071799) was conducted according to the Declaration of Helsinki, approved by the institutional review boards of the participating study sites, and all patients provided written informed consent. Eligible patients had International Prognostic Scoring System-defined intermediate-2- or high-risk MDS and French-American-British–defined refractory anemia with excess blasts (RAEB), RAEB in transformation, or chronic myelomonocytic leukemia with $\geq$10% bone marrow blasts and a white blood cell count $<13 \times 10^9$/L.[1,11] Patients with an Eastern Cooperative Oncology Group (ECOG) performance status score of 0 to 2 and estimated life expectancy of $\geq$3 months were included, whereas those with treatment-related MDS, prior azacitidine, decitabine, or chemotherapy treatment, or planned allogeneic stem cell transplantation were ineligible.

The protocol specified that azacitidine should be administered subcutaneously at a dose of 75 mg/m^2 daily for the first 7 days of each 28-day cycle, and for at least 6 cycles. Treatment was to be continued until disease progression or unacceptable toxicity was observed. After treatment discontinuation, all patients were followed until death or study completion. All patients could receive best supportive care, including the use of granulocyte colony-stimulating factors for neutropenic infection, but the use of erythropoiesis-stimulating agents was prohibited.

Blood counts and bone marrow biopsies were analyzed according to the International Working Group 2000 criteria.[12] Blood counts were performed weekly for the first 2 cycles and every other week thereafter. Bone marrow aspirates and biopsies were performed as required, or at a minimum of every 16 weeks. Response to treatment was classified according to International Working Group 2000 criteria[12]: complete response (CR) was investigator-assessed; partial response (PR) was investigator-assessed; and major or minor hematological improvement was programmatically derived. Median time to first response was evaluated from date of randomization, and median time from first to best response was evaluated from the date of the first response to best response. For these endpoints, actual cycles were calculated by dividing the number of days on treatment by 28 days, while excluding dose interruptions. Red blood cell (RBC) transfusion independence was defined as the absence of RBC transfusions for $\geq$56 consecutive days during the treatment period. Median times and the corresponding 95% confidence intervals (CIs) were estimated using Kaplan-Meier[13] methods stratified by International Prognostic Scoring System and French-American-British classification. Median time to RBC transfusion independence and the median duration of RBC transfusion independence were analyzed using patients who were transfusion dependent at baseline. All analyses were performed using SAS 9.13.

RESULTS

Of the 179 patients who were assigned to azacitidine treatment, 91 (51%) achieved a response (CR, PR, or hematological improvement). The baseline characteristics of these 91 responders are shown in Table 1. Median patient age was 69 years (range, 42-83 years). Most patients were male (78%) and had RAEB (66%), an ECOG performance status score of 0 to 1 (95%), and multiple cytopenias (97%). In addition, 57 (63%) patients were RBC transfusion dependent at baseline.

Responding patients (those who achieved CR, PR, or hematological improvement) received a median of 14 cycles of azacitidine (range, 2-30; Table 2). The median

Table 1. Baseline Demographics of Patients Who Achieved a Complete Response, Partial Response, or Hematological Improvement with Azacitidine (n = 91)

Characteristic	No. of Patients (%)
Sex	
Male	71 (78)
Female	20 (22)
French-American-British classification	
Refractory anemia with excess blasts	60 (66)
Refractory anemia with excess blasts in transformation	24 (26)
Chronic myelomonocytic leukemia	4 (4)
Not per protocol[a]	2 (2)
Indeterminate	1 (1)
Eastern Cooperative Oncology Group performance status score	
0	47 (52)
1	39 (43)
2	4 (4)
Missing	1 (1)
Duration of myelodysplastic syndromes, y	
Mean ± standard deviation	1.2 ± 2.0
Median (range)	0 (0-10)
Number of cytopenias	
1	3 (3)
2	30 (33)
3	58 (64)
International Prognostic Scoring System	
Intermediate-1 risk	3 (3)
Intermediate-2 risk	38 (42)
High risk	44 (48)
Not applicable[a]	2 (2)
Indeterminate	4 (4)
International Prognostic Scoring System cytogenetics	
Good	43 (47)
Intermediate	17 (19)
Poor	26 (29)
Missing	5 (6)
Red blood cell transfusion dependence	57 (63)

[a] Includes 1 patient with acute myelogenous leukemia and 1 patient with myeloproliferative disease.

azacitidine cycle length was 28 days (interquartile range, 20-189 days); 651 (54%) of the 1205 cycle lengths were 28 days, 320 (27%) were 29 to 35 days, and 234 (19%) were longer than 35 days. Cycle delays with azacitidine occurred primarily because of the extended time necessary for nadir and hematological recovery.

The median number of cycles to any first response (CR, PR, or hematological improvement) was 2 (range, 1-16; Fig. 1); 1 patient achieved a first response of CR after 2 cycles of therapy. The median number of cycles to a first response of PR (n = 6) or hematological improvement (n = 84) was 4 (range, 2-8) and 2 (range, 1-16), respectively. Overall, 91% of responding patients achieved their first response within 6 cycles. Of the remaining 9% of patients, all achieved their first response by 12 cycles, except 1 patient who had first response at Cycle 16. Also of these 9% of patients, 1 achieved a PR and 7 a hematological improvement, including 2 patients who later improved to CR and PR, respectively. The first response achieved was also the best response in 52% of patients. Of the remaining 48% of patients, continued azacitidine treatment after initial response led to achievement of a higher response category (Fig. 2). In these 48% of patients with an improved response after a first response, the median number of additional cycles from first response to best response was 3 (range, 1-11). By Cycle 12 of treatment with azacitidine, 92% of responding patients had achieved their best response. None of the 6 patients who achieved a PR as the first response improved to CR; however, 44 (52%) of 84 patients who achieved a hematological improvement as their first response went on to achieve either a CR or PR as their best response. Among 30 patients who ultimately achieved a CR, median time from first to best response was 3.5 cycles (95% CI, 3.0-6.0; Table 3). In the 21 patients who achieved a best response of PR, the median time from first to best response was 3.0 cycles (95% CI, 1.0-3.0). After the maximum level of response was achieved, azacitidine treatment was continued for a median of 8 additional cycles (range, 0-27).

Table 2. Number of Azacitidine Cycles Received by Patients Who Achieved Complete Response, Partial Response, or Hematological Improvement With Azacitidine (n = 91)

International Working Group 2000 Response	No. (%)	Azacitidine Cycles	
		Median	Range
Overall	91 (100)	14.0	2-30
Complete response	30 (33.0)	16.5	5-30
Partial response	21 (23.1)	14.0	2-27
Hematological improvement	40 (44.0)	11.5	3-25

A total of 57 responders in this analysis were RBC transfusion dependent at baseline (ie, had transfusions during the 56 days before randomization). Of these, 88% achieved RBC transfusion independence at some stage during treatment with azacitidine. Median time to RBC transfusion independence was 1.6 months (95% CI, 1.2-2.1), and the median duration of RBC transfusion independence was 13.0 months (95% CI, 7.2-20.9).

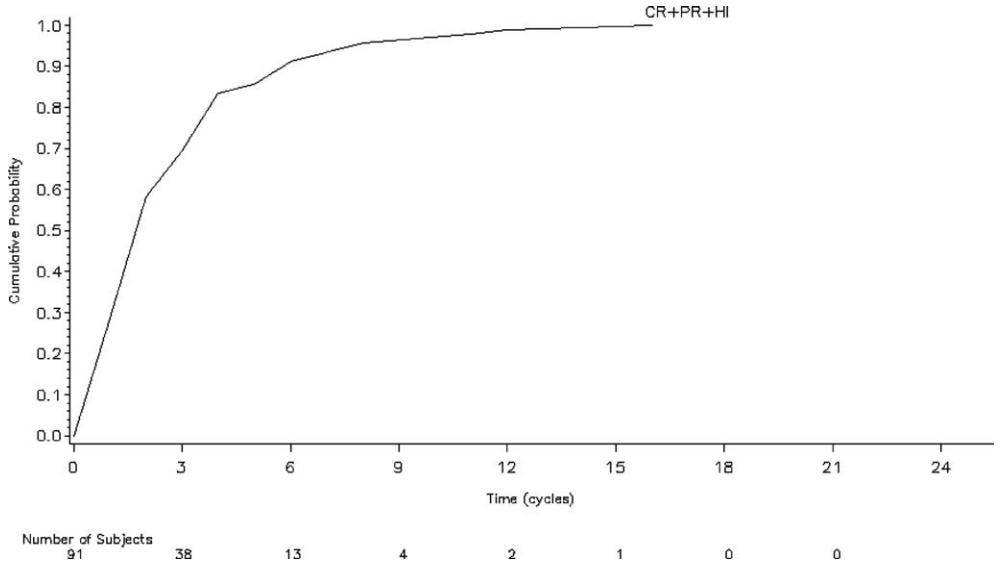

Figure 1. Time to first response (complete response [CR], partial response [PR], or hematological improvement [HI]) in patients who achieved a response during treatment with azacitidine is shown.

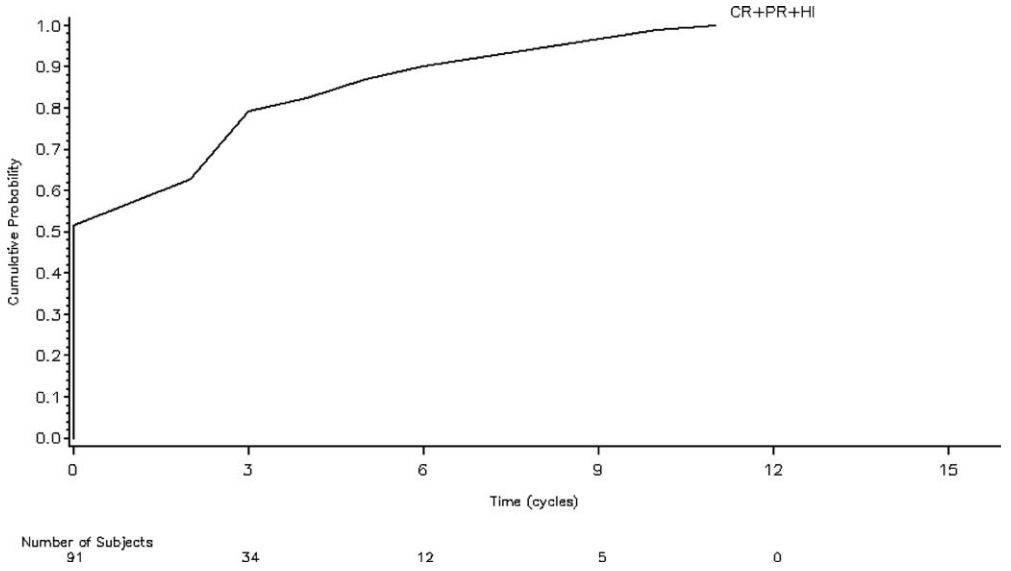

Figure 2. Time to best response after first response in patients treated with azacitidine is shown. The vertical line on the y-axis represents the 52% of patients whose first response was their best response. The remaining 48% of patients had an improvement in their first response with continued azacitidine therapy; they all improved from a first response of hematological improvement (HI) to either partial response (PR) or complete response (CR).

DISCUSSION

The purpose of this secondary analysis of the AZA-001 trial was to provide a better understanding of the timing and pattern of response to azacitidine therapy in patients with higher-risk MDS. Considering that 91% of first responses occurred within 6 cycles, the data from this study suggest that outcomes with azacitidine should be evaluated after a minimum of 6 cycles of treatment. In the remaining 9% of patients with stable disease at 6 cycles, continued therapy beyond 6 cycles resulted in achievement of a first response.

This analysis shows that although most responses occurred within 6 cycles, continued azacitidine therapy

Table 3. Time From First to Best Response in Patients Treated With Azacitidine

Subgroup	No.	Median (range) Time From First to Best Response, Cycles	95% CI for Median
Patients who achieved a best response of partial response	21	3.0 (0.0-6.0)	1.0-3.0
Patients who achieved a best response of complete response	30	3.5 (0.0-11.0)	3.0-6.0

CI indicates confidence interval.

led to a further improvement in response category in almost half (48%) of all responders with a median of 3 additional cycles, and that 92% of patients achieved their best response by Cycle 12. In a randomized phase 3 trial conducted by the US Cancer and Leukemia Group B, which compared azacitidine with best supportive care, most responses occurred during the third or fourth month of azacitidine therapy.[7] A subsequent combined analysis of 3 sequential azacitidine Cancer and Leukemia Group B studies showed that first response to azacitidine occurred after a median of 3 cycles.[8] The phase 3 Cancer and Leukemia Group B study also showed that 90% of responses occurred within the first 6 cycles of treatment and that best response generally occurred 2 cycles after the first response—all of which is consistent with the current findings. Taken together, these data suggest that although some effects of azacitidine manifest promptly, additional courses are usually necessary before best response is achieved. Therefore, continuing azacitidine therapy offers the best chance of enhanced benefit if treatment is tolerated and there is no evidence of disease progression.[10] This information on the timing of responses should be useful in monitoring the progress of a patient during treatment.

Possible explanations for the observed pattern of response to azacitidine may lie in its modulatory effects on the MDS clone. The cytotoxic and hypomethylating effects of azacitidine depend on the incorporation of its metabolite into newly synthesized RNA and DNA.[5,14-17] Multiple cell cycles may have to occur before sufficient drug is incorporated to mediate sustained alterations in gene expression.[7,8] Pharmacokinetic studies demonstrate a short plasma half-life for azacitidine, making it difficult to explain how the rapid hypomethylating effect can occur in cell populations where few cells are in cell cycle S-phase.[18] However, Gore et al[9] showed that clinical response to azacitidine in patients with MDS correlated with pharmacokinetic measures of drug exposure and azacitidine-induced hypomethylation, providing some of the first evidence to directly link the drug target effects of azacitidine with clinical outcomes. Moreover, after the first cycle of treatment, they observed heterogeneous methylation of alleles in bone marrow cells and an incomplete demethylation pattern in 3 responding patients. This observation suggests that azacitidine-induced hypomethylation occurs progressively within the MDS clone and that maximal demethylation may require multiple exposures to the drug.[9] Furthermore, once azacitidine treatment is stopped, aberrant promoter methylation and gene silencing return, suggesting that prolonged treatment will be needed to maintain inhibition of DNA methylation.[19]

Although Fenaux et al[3] demonstrated a major survival benefit with azacitidine compared with conventional care regimens, the CR rates achieved with azacitidine in this setting have been modest, leading to the hypothesis that attainment of a CR is not essential for the survival benefit observed with azacitidine.[3,8,20,21] Improved survival has also been noted despite the persistence of the MDS-associated cytogenetic clone or the emergence of new abnormal cytogenetic clones in some patients.[7,20] Therefore, azacitidine may affect the differentiation and growth of the MDS clone without necessarily eradicating it, suggesting that repetitive and prolonged exposure to azacitidine may be necessary for both the initial effects and the subsequent augmentation of response.

In summary, our findings demonstrate that, although some MDS patients respond rapidly to azacitidine therapy (eg, in early treatment cycles), continued azacitidine therapy results in a qualitative increase in response quality in almost half of the responders. These data support the continued use of azacitidine in responding patients as long as the patient continues to benefit.

CONFLICT OF INTEREST DISCLOSURES

The study was funded by Celgene Corporation. The authors received editorial/writing support provided by Excerpta Medica in the preparation of the manuscript, funded by Celgene Corporation. The authors are fully responsible for content and editorial decisions for this article. Arlene Swern, PhD and David McKenzie, MS of Celgene Corporation provided statistical support to the authors during manuscript development. We thank Jay Backstrom, MD and C. L. Beach, PharmD from Celgene Corporation for their assistance with manuscript development. Financial disclosures: L. R. Silverman: honoraria, Celgene; P. Fenaux: honoraria, Amgen, Celgene, Cephalon, GSK, Johnson & Johnson, Roche, and MSD; research funding, Celgene and Roche; G. J. Mufti: honoraria,

speaker's bureau, and research funding, Amgen and Celgene; V. Santini: honoraria, Celgene and Novartis; E. Hellström-Lindberg: consultancy and research funding, Celgene; N. Gattermann: research funding and honoraria, Celgene and Novartis; G. Sanz: advisory committee member, honoraria, and research funding, Celgene; A. F. List: research funding and honoraria, Celgene; S. D. Gore: consultancy, equity ownership, and research funding, Celgene; J. F. Seymour: research funding, honoraria, speaker's bureau, and advisory committee member, Celgene.

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. Barrett AJ, Savani BN. Allogeneic stem cell transplantation for myelodysplastic syndrome. *Semin Hematol*. 2008;45: 49-59.
3. Fenaux P, Mufti GJ, Hellstrom-Lindberg E, et al. Efficacy of azacitidine compared with that of conventional care regimens in the treatment of higher-risk myelodysplastic syndromes: a randomised, open-label, phase III study. *Lancet Oncol*. 2009;10:223-232.
4. Kornblith AB, Herndon JE Jr, 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.
5. D'Alò F, Voso MT, Leone G. Profile of azacitidine. *Therapy*. 2005;2:717-731.
6. Santini V, Kantarjian HM, Issa JP. Changes in DNA methylation in neoplasia: pathophysiology and therapeutic implications. *Ann Intern Med*. 2001;134:573-586.
7. 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.
8. Silverman LR, McKenzie DR, Peterson BL, et al. Further analysis of trials with azacitidine in patients with myelodysplastic syndrome: studies 8421, 8921, and 9221 by the Cancer and Leukemia Group B. *J Clin Oncol*. 2006;24: 3895-3903.
9. Gore SD, Baylin S, Sugar E, et al. Combined DNA methyltransferase and histone deacetylase inhibition in the treatment of myeloid neoplasms. *Cancer Res*. 2006;66: 6361-6369.
10. Garcia-Manero G. Improving survival in myelodysplastic syndromes. *Lancet Oncol*. 2009;10:200-201.
11. Bennett JM, Catovsky D, Daniel MT, et al. Proposals for the classification of the myelodysplastic syndromes. *Br J Haematol*. 1982;51:189-199.
12. Cheson BD, Bennett JM, Kantarjian H, et al. Report of an international working group to standardize response criteria for myelodysplastic syndromes. *Blood*. 2000;96:3671-3674.
13. Kaplan EL, Meier P. Nonparametric estimation for incomplete observations. *J Am Stat Assoc*. 1958;53:457-481.
14. Creusot F, Acs G, Christman JK. Inhibition of DNA methyltransferase and induction of Friend erythroleukemia cell differentiation by 5-azacytidine and 5-aza-2′-deoxycytidine. *J Biol Chem*. 1982;257:2041-2048.
15. Taylor SM, Jones PA. Multiple new phenotypes induced in 10T1/2 and 3T3 cells treated with 5-azacytidine. *Cell*. 1979;17:771-779.
16. Khan R, Schmidt-Mende J, Karimi M, et al. Hypomethylation and apoptosis in 5-azacytidine-treated myeloid cells. *Exp Hematol*. 2008;36:149-157.
17. Stresemann C, Lyko F. Modes of action of the DNA methyltransferase inhibitors azacytidine and decitabine. *Int J Cancer*. 2008;123:8-13.
18. Marcucci G, Silverman L, Eller M, Lintz L, Beach CL. Bioavailability of azacitidine subcutaneous versus intravenous in patients with the myelodysplastic syndromes. *J Clin Pharmacol*. 2005;45:597-602.
19. Herman JG, Baylin SB. Gene silencing in cancer in association with promoter hypermethylation. *N Engl J Med*. 2003;349:2042-2054.
20. Najfeld V, Scalise A, Odchimer-Reissig R, Silverman LR. The modulating effects of azacitidine (Aza C) on the cytogenetically tracked MDS clone impact survival [abstract]. *Blood*. 2004;104. Abstract 1429.
21. List AF, Fenaux P, Mufti GJ, et al. Effect of azacitidine (AZA) on overall survival in higher-risk myelodysplastic syndromes (MDS) without complete remission [abstract]. *J Clin Oncol.* 2008;26(suppl). Abstract 7006.