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ORIGINAL REPORT

Further Analysis of Trials With Azacitidine in Patients With Myelodysplastic Syndrome: Studies 8421, 8921, and 9221 by the Cancer and Leukemia Group B

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ABSTRACT

Purpose
Within the last two decades, a new understanding of the biology of myelodysplastic syndrome (MDS) has developed. With this understanding, new classification systems, such as the WHO diagnostic criteria, and the International Prognostic Scoring System and response criteria guidelines reported by the International Working Group (IWG) have been developed. We report the combined results of three previously reported clinical trials (n = 309) with azacitidine using the WHO classification system for MDS and acute myeloid leukemia (AML) and IWG criteria for response.

Patients and Methods
Data from three sequential Cancer and Leukemia Group B trials with azacitidine were recollected and reanalyzed as part of the New Drug Application process. The trials were conducted with either intravenous or subcutaneous azacitidine (75 mg/m^2/d for 7 days every 28 days).

Results
Complete remissions were seen in 10% to 17% of azacitidine-treated patients; partial remissions were rare; 23% to 36% of patients had hematologic improvement (HI). The median number of cycles to first response was three, and 90% of responses were seen by cycle 6. Using current WHO criteria, 103 patients had AML at baseline; 35% to 48% had HI or better responses. The median survival time for the 27 AML patients randomly assigned to azacitidine was 19.3 months compared with 12.9 months for the 25 patients assigned to observation. Furthermore, azacitidine did not increase the rate of infection or bleeding above the rate caused by underlying disease.

Conclusion
Azacitidine provides important clinical benefits for patients with high-risk MDS.

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INTRODUCTION

In 1984, the Cancer and Leukemia Group B (CALGB) began a series of clinical trials with azacitidine (Vidaza; Pharmion Corporation, Overland Park, KS) in patients with myelodysplastic syndrome (MDS).[1-4] These studies and other supportive data culminated in the 2004 US Food and Drug Administration approval of azacitidine for treatment of symptomatic patients with MDS. During the intervening two decades, a greater understanding of the biology of myelodysplasia has evolved, along with a new classification system developed by WHO that more clearly distinguishes MDS from acute myeloid leukemia (AML) and from chronic myeloproliferative disorders.[5,6] In addition, an International Working Group (IWG) sponsored by the National Cancer Institute (NCI) has published new response criteria for evaluation of new treatments for MDS.[7,8] As part of the New Drug Application process, Pharmion recollected and reanalyzed the CALGB data, including expert pathology review of blood and bone marrow slides. Some of the CALGB data from these three trials was previously published using the protocol-specified diagnostic and response criteria. Here, we report the combined results of a reanalysis using the WHO classification for MDS and AML and the IWG criteria for response in MDS.

PATIENTS AND METHODS

Data Collection

For the reanalysis, data were recollected from patients enrolled onto CALGB Protocols 8421, 8921, and 9221.[1-3] A comprehensive retrospective collection and reverification of all clinical data in the original protocols

were conducted. A complete safety database was collected, including additional data for azacitidine and data for the best supportive care (observation) arm of Protocol 9221; in the initial report by Silverman et al,[3] safety data were only collected and reported for azacitidine-treated patients. To validate the reliability of bone marrow slides and peripheral-blood films used for diagnosis and response, an independent blinded review was performed retrospectively (John M. Bennett) in addition to the previously conducted prospective local and central pathology reviews (Frederick R. Davey and Rose Ruth Ellison). All recollected data were entered into a new database held by Pharmion; our analyses were conducted separately from those reported in Silverman et al.[1-3]

Patient Selection

All patients enrolled onto CALGB Protocols 8421, 8921, and 9221 were included in our analyses. Consent forms were approved annually by local institutional review boards; each patient provided written consent. Detailed descriptions of the study designs of these three studies have been previously published.[1-3] Because Protocol 9221 included a comparative arm of best supportive care (observation), further analyses (eg, time to response, transfusion independence, and safety comparisons) were conducted.

Treatment Regimen

Although the route of administration was either continuous intravenous infusion (Protocol 8421) or subcutaneous (Protocols 8921 and 9221), the azacitidine dose investigated was 75 mg/m^2/d for 7 days, repeated in a 28-day cycle, in all three studies. No dose adjustments at inception of treatment were made for pre-existing cytopenias despite severity. Therapy was continued for three cycles after complete remission (CR) or until progressive disease or toxicity in patients with partial remission (PR) or hematologic improvement (HI). After 4 months, patients in the observation group could cross over to azacitidine after evidence of disease progression.[3]

Definitions

Treatment groups. In Protocol 9221, patients randomly assigned to azacitidine plus best supportive care were referred to as the azacitidine arm (n = 99); patients randomly assigned to best supportive care alone were referred to as the observation arm (n = 92). Patients randomly assigned to best supportive care alone but who did not cross over to azacitidine were referred to as the observation-only arm (n = 41); patients randomly assigned to best supportive care and who received azacitidine after cross over from the observation group were referred to as the azacitidine after observation arm (n = 51).

Response criteria. General differences between CALGB and IWG response criteria for MDS included duration of response (CALGB: ≥ 4 weeks; IWG: ≥ 8 weeks), normal target peripheral-blood values (CALGB: different criteria for hemoglobin targets for males and females; IWG: same criteria for males and females), and generally more stringent criteria for lineage response with the CALGB criteria. The IWG criteria for improvement differentiate major and minor responses by peripheral-blood values[7] but were combined in our analyses to give one overall calculation of improvement for patients.

WHO AML response rates. To evaluate responses in patients with MDS who were determined after expert pathology review to have AML at study entry, we applied the WHO classification for AML and removed refractory anemia with excess blasts (RAEB) in transformation (RAEB-T) from the MDS classification.[5,6] Therefore, our use of the WHO definition of AML includes patients previously defined as RAEB-T and AML by French-American-British criteria.

Transfusion independence. On the basis of IWG criteria, transfusion independence was defined as a transfusion-free period of ≥ 56 days (8 weeks) occurring anytime after random assignment. Transfusion dependence at baseline was defined as ≥ one transfusion within 90 days before random assignment.[8]

Adverse events. Any event occurring during the study not present at baseline or that worsened from baseline was documented as an adverse event (AE). All patients who received ≥ one dose of azacitidine were included in the azacitidine group as part of the safety analyses. For observation patients who crossed over, AEs that occurred during the period of observation were included in the observation safety analyses; any event that occurred after exposure to azacitidine was considered an azacitidine event and included in the azacitidine safety analyses.

Statistical Methods

For response rate analysis, the azacitidine group was compared with the observation-only group. For the survival analysis of patients with WHO AML, patients were analyzed as randomly assigned. If a patient was randomly assigned to the observation group, then all time to event data for that patient were analyzed in the observation group even if the patient later crossed over to azacitidine treatment. Safety analyses were performed on an as-treated basis.

Because the studies were conducted before availability of the IWG standardized response criteria for MDS, we retrospectively applied the IWG response criteria[7] for CR, PR, and HI. Time to response was measured from the date of random assignment (Protocol 9221) or entry onto the phase II studies (Protocols 8421 and 8921) to the date of the event.

The original 9221 study was not powered for overall survival in the subgroup of WHO AML patients. However, overall survival times were compared using the exact log-rank test. Statistical comparisons of groups in the proportion of patients achieving transfusion independence were performed using Fisher's exact test.

Numeric laboratory values were defined by a specific NCI Common Toxicity Criteria (CTC) grade, ranging from 0 to 4. The maximum NCI CTC grade within a cycle was used to represent that cycle. Baseline was determined as the maximum grade before the date of random assignment or, if no value was available before random assignment, as the earliest value after random assignment up to and including the day of the first dose of azacitidine for azacitidine patients, and baseline was determined as the maximum grade on the day of random assignment for observation patients.

To determine whether azacitidine was associated with an increase in either infection or bleeding, we examined the rates (in patient-years) of infection or bleeding AEs associated with azacitidine therapy compared with the rates seen with the underlying disease (on the observation arm). Summaries are inclusive of nonserious and serious AEs.

RESULTS

Patient Characteristics in Protocols 8421, 8921, and 9221

Among the three studies, 268 patients were treated with azacitidine, of whom 220 patients were treated with subcutaneous azacitidine and 48 were treated with intravenous azacitidine; 41 patients received best supportive care on the observation arm of Protocol 9221 (Table 1). The majority of all 309 patients registered or randomly assigned to azacitidine or observation were male (68%, 210 of 309 patients), white (94%, 292 of 309 patients), and ≥ 65 years of age (61%, 188 of 309 patients). The distribution of MDS subtypes at baseline diagnosis was similar among the azacitidine and observation groups in Protocol 9221. The percentage of patients with AML using WHO criteria was 52% in Protocol 8421, 37% in Protocol 8921, and 27% in the azacitidine arm and 27% (25 of 92 patients) in the observation arm of Protocol 9221.

Response in Protocols 8421, 8921, and 9221 Based on IWG Response Criteria for MDS

After applying the IWG response criteria to Protocols 8421, 8921, and 9221, response rates in the azacitidine groups were consistent across the studies, with between 40% and 47% of patients demonstrating a response. Ten percent to 17% of patients achieved a CR; PR was rare; and 23% to 36% of patients had HI (Table 2). Median duration of response was 13.1 months (range, 2 to 165.8+ months).

Patients With WHO AML

Because the previously discussed IWG response rates included some patients with an adjudicated diagnosis of AML, these patients were reviewed separately after redefining the AML diagnosis based on

Table 1. Baseline Demographics and Disease Characteristics in Protocols 8421, 8921, and 9221

Demographic	Protocol 8421: IV Azacitidine (n = 48)		Protocol 8921: SC Azacitidine (n = 70)*		Protocol 9221					
					SC Azacitidine† (n = 99)		Observation Only‡ (n = 41)		SC Azacitidine After Observation (n = 51)	
	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%
Sex										
Male	31	65	47	67	72	73	29	71	31	61
Female	17	35	23	33	27	27	12	29	20	39
Race										
White	48	100	66	94	93	94	38	93	47	92
Black	0	0	2	3	1	1	0	0	1	2
Hispanic	0	0	1	1	3	3	3	7	2	4
Asian	0	0	0	0	2	2	0	0	1	2
Other	0	0	1	1	0	0	0	0	0	0
Age, years										
No. of Patients	48		66		99		41		51	
Median	65.0		66.0		69.0		68.5		66.0	
Range	35-81		23-82		31-92		35-88		46-82	
Missing	0	0	4	6	0	0	1	2	0	0
< 65	21	44	26	37	36	36	16	39	17	33
65-74	24	50	24	34	39	39	11	27	22	43
≥ 75	3	6	16	23	24	24	13	32	12	24
Diagnosis at study entry§										
RA	0	0	7	10	16	16	3	7	17	33
RARS	0	0	4	6	6	6	2	5	5	10
RAEB	23	48	19	27	38	38	18	44	14	27
RAEB-T	24	50	16	23	17	17	7	17	9	18
CMMoL	0	0	14	19	12	12	6	15	2	4
AML	1	2	10	19	10	10	5	12	4	8
WHO AML‖	25	52	26	37	27	27	12	29	13	26
Performance status										
0, normal	15	31	19	27	35	35	13	32	13	26
1, fatigue	13	27	33	47	34	34	15	37	24	47
2, impaired	7	15	5	7	8	8	4	10	2	4
3, bed rest	3	6	0	0	1	1	0	0	0	0
Unknown/not performed	10	21	13	19	21	21	9	22	12	24
Transfusion product used within 3 months before study entry										
Any transfusion product	43	90	52	74	70	71	23	56	36	71
RBCs, packed	41	85	51	73	66	67	21	51	34	67
Hetastarch	0	0	0	0	0	0	0	0	1	2
Plasma protein fraction, human	0	0	0	0	1	1	0	0	0	0
Platelets,	16	33	20	29	15	15	7	17	5	10
Unknown	3	6	0	0	2	2	1	2	1	2

Abbreviations: IV, intravenous; SC, subcutaneous; RA, refractory anemia; RARS, refractory anemia with ringed sideroblasts; RAEB, refractory anemia with excess blasts; RAEB-T, refractory anemia with excess blasts in transformation; CMMoL, chronic myelomonocytic leukemia; AML, acute myeloid leukemia; WHO AML, WHO classification of AML.

*Two additional patients were registered (n = 72) but never received study medication.

†Patients randomly assigned to azacitidine.

‡Patients randomly assigned to observation who did not cross over to azacitidine.

§No patients were enrolled with a diagnosis of RA, RARS, or CMMoL in Protocol 8421 because this study only allowed patients with RAEB or RAEB-T myelodysplastic syndrome subtypes for enrollment.

‖Includes patients with RAEB-T and AML diagnosis after central review.

WHO criteria. Between 35% and 48% of patients in the azacitidine groups with WHO AML in Protocols 8421, 8921, or 9221 experienced CR, PR, or HI (Table 3). Among the 33 WHO AML responders in the three studies, the median duration of response was 7.3 months (range, 2.2 to 25.9 months).

In Protocol 9221, 7% of patients with WHO AML in the azacitidine group achieved CR or PR compared with 0% in the observation-only group. Median survival time for the 27 WHO AML patients in the azacitidine group was 19.3 months compared with 12.9 months for the 25 WHO AML patients randomly assigned to observation. Of 13 patients with WHO AML at study entry who crossed over to azacitidine, one patient who was on the observation arm for 5.2 months achieved PR, and one patient who was on the observation arm for 4.1 months achieved HI. Of the 11 patients who

Table 2. Best Response for All Patients Using IWG Response Criteria for MDS in Protocols 8421, 8921, and 9221

IWG Response	Protocol 8421: IV Azacitidine (n = 48)		Protocol 8921: SC Azacitidine (n = 70)		Protocol 9221						Protocols 8921 and 9221: SC Azacitidine (n = 169)	
					SC Azacitidine* (n = 99)		Observation Only† (n = 41)		SC Azacitidine After Observation (n = 51)			
	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%
CR	7	15	12	17	10	10	0	0	3	6	22	13
PR	1	2	0	0	1	1	0	0	2	4	1	1
HI‡	13	27	16	23	36	36	7	17	13	25	52	31
Erythroid response, major	10	21	11	16	22	22	1	2	8	16	33	20
Erythroid response, minor	2	4	3	4	8	8	4	10	4	8	11	7
Platelet response, major	9	19	6	9	21	21	2	5	3	6	27	16
Platelet response, minor	0	0	2	3	3	3	0	0	1	2	5	3
Neutrophil response, major	2	4	0	0	8	8	1	2	2	4	8	5
Neutrophil response, minor	0	0	0	0	0	0	0	0	0	0	0	0
Overall: CR + PR + HI‡	21	44	28	40	47	47	7	17	18	35	75	44

Abbreviations: IWG, International Working Group; MDS, myelodysplastic syndrome; IV, intravenous; SC, subcutaneous; CR, complete remission; PR, partial remission; HI, hematologic improvement.
*Patients randomly assigned to azacitidine.
†Patients randomly assigned to observation who did not cross over to azacitidine.
‡Patients with HI (major or minor) were counted only once in the overall response.

crossed over to azacitidine but had no response, the median time to cross over was 3.3 months after study entry (range, 1.4 to 10.3 months). Among WHO AML patients who were transfusion independent at baseline, duration of transfusion independence was significantly longer for patients in the azacitidine group compared with the observation group for both RBC (azacitidine: 14.7 months, n = 8; observation: 4.8 months, n = 9; $P = .02$) and platelets (azacitidine: 13 months, n = 13; observation: 4.5 months, n = 18; $P = .004$).

Time to Response and Duration of Response Based on IWG Response Criteria in Protocols 8421, 8921, and 9221

In all three studies, the median number of cycles from the first treatment with azacitidine to any response (CR, PR, or HI) was three cycles (range, one to 17 cycles). Although 75% of the responders achieved a response by cycle 4, the other 25% achieved a response as late as cycle 17. The majority of responders (90%) achieved a response

Table 3. Best Response for All Patients With AML (WHO) in Protocols 8421, 8921, and 9221 Using IWG Response Criteria for MDS

IWG Response	Protocol 8421: IV Azacitidine (n = 25)		Protocol 8921: SC Azacitidine (n = 26)		Protocol 9221						Protocols 8921 and 9221: SC Azacitidine (n = 53)	
					SC Azacitidine* (n = 27)		Observation Only† (n = 12)		SC Azacitidine After Observation (n = 13)			
	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%	No. of Patients	%
CR	3	12	3	12	2	7	0	0	0	0	5	9
PR	1	4	0	0	0	0	0	0	1	8	0	0
HI‡	8	32	6	23	8	30	0	0	1	8	14	26
Erythroid response, major	5	20	4	15	6	22	0	0	1	8	10	19
Erythroid response, minor	1	4	1	4	0	0	0	0	0	0	1	2
Platelet response, major	5	20	1	4	6	22	0	0	0	0	7	13
Platelet response, minor	0	0	1	4	1	4	0	0	0	0	2	4
Neutrophil response, major	0	0	0	0	1	4	0	0	1	8	1	2
Neutrophil response, minor	0	0	0	0	0	0	0	0	0	0	0	0
Overall: CR + PR + HI‡	12	48	9	35	10	37	0	0	2	15	19	36

Abbreviations: AML, acute myeloid leukemia; IWG, International Working Group; MDS, myelodysplastic syndrome; IV, intravenous; SC, subcutaneous; CR, complete remission; PR, partial remission; HI, hematologic improvement.
*Patients randomly assigned to azacitidine.
†Patients randomly assigned to observation who did not cross over to azacitidine.
‡Patients with HI (major or minor) were counted only once in the overall response.

Table 4. Proportion and Duration of RBC Transfusion-Free* Periods in Responders Who Were Previously Transfusion-Dependent Patients in Protocol 9221

Treatment and FAB Classification†	No. of Patients	Patients Independent After Random Assignment		Duration of Transfusion Independence (months)	
		No.	%	Median	Range
RBC transfusion-dependent patients at baseline‡ who achieved CR, PR, or HI	45	36	80		
Azacitidine	29	25	86	9.0	2.0-63.0
RA	6	6	100	13.0	2.0-63.0
RARS	2	1	50	17.3	
RAEB	10	9	90	8.6	2.5-33.4
RAEB-T	3	3	100	9.4	6.8-10.3
CMMoL	5	3	60	8.4	4.0-10.0
AML	3	3	100	10.9	3.8-25.8
Observation only	5	1	20	2.3	—
RA	0	0	0		
RARS	0	0	0		
RAEB	3	1	33	2.3	—
RAEB-T	0	0	0		
CMMoL	2	0	0		
AML	0	0	0		
Azacitidine after observation	11	10	91	9.6	2.0-88.4
RA	3	3	100	6.7	5.3-14.8
RARS	1	1	100	12.4	—
RAEB	5	5	100	6.6	3.0-88.4
RAEB-T	2	1	50	12.9	—
CMMoL	0	0	0		
AML	0	0	0		

NOTE. Comparison of proportion of patients achieving a transfusion-free period using Fisher's two-sided exact test: azacitidine *v* observation only, $P = .007$.
Abbreviations: FAB, French-American-British; CR, complete remission; PR, partial remission; HI, hematologic improvement; RA, refractory anemia; RARS, refractory anemia with ringed sideroblasts; RAEB, refractory anemia with excess blasts; RAEB-T, refractory anemia with excess blasts in transformation; CMMoL, chronic myelomonocytic leukemia; AML, acute myeloid leukemia.
*Transfusion-free period defined as ≥ 56 days.
†On the basis of adjudicated review at baseline and those patients with blasts > 20%, the No. of WHO AML patients included six in the azacitidine group, zero in the observation group, and two in the azacitidine after observation group.
‡Transfusion administered within 90 days before random assignment.

by cycle 6. Best response was observed, on average, two cycles after initial response and lasted for a median of five cycles.

Transfusion Independence in Protocol 9221

Of the 191 patients enrolled onto Protocol 9221, 121 (63%) were RBC transfusion dependent at baseline. Of these RBC transfusion–dependent patients, 45 showed a response (CR, PR, or HI). A total of 36 of the responders became transfusion independent, of whom 35 had received azacitidine (Table 4). Among all RBC transfusion–dependent patients treated initially with azacitidine, 45% (29 of 65 patients) achieved RBC transfusion independence. A similar trend was noted for the 27 patients who were platelet transfusion dependent at baseline. Furthermore, among all 72 overall responders, platelet transfusion independence was achieved in all eight patients randomly assigned to azacitidine and in both patients who received azacitidine after cross over.

The median duration of RBC transfusion independence was 9 months in patients with CR, PR, or HI who were transfusion dependent before receiving azacitidine (n = 25) compared with 2.3 months for observation-only patients (n = 1; Table 4). A similar trend was noted for platelet transfusion independence, for which the median duration of independence was 6.3 months in the azacitidine group (n = 8) compared with 2.4 months for the observation-only group (n = 1).

Safety Results in Protocol 9221

Hematology. Mean times to nadir values (across all cycles) for RBC, hemoglobin, WBC, absolute neutrophils, and platelets were between 15 and 16 days. On the basis of the overall frequency of time to nadir within the cycle (days 1 to 7, 8 to 14, 15 to 21, or > 21), hematology values tended to reach nadir values during the second or third week of the cycle (Table 5).

Most patients had NCI CTC grade 0 to 2 hematology values at baseline (Table 6). Many patients in both treatment groups had shifts

Table 5. Onset of Hematology Nadir Values in Cycle Day (all cycles combined) in Protocol 9221 for All Azacitidine Patients (N = 150)

Hematology Value	No. of Patients	Cycle Day of Onset of Nadir Values		
		25th Percentile	Median	75th Percentile
Hemoglobin	149	11	15	19
WBCs	149	13	16	20
Absolute neutrophils	143	12	17	22
Platelets	148	13	15	18

NOTE. Table includes all patients exposed to azacitidine, including patients who crossed over to azacitidine from observation.

Table 6. Summary of Hematology Shifts by Maximum NCI CTC Grade Criteria (hematology) in Protocol 9221

Maximum NCI CTC Grade Baseline Value	Postbaseline* Maximum NCI CTC Grade												
	All Azacitidine Patients† (n = 150)							Observation (n = 92)					
	No. of Patients	Grades 0-2		Grade 3		Grade 4		No. of Patients	Grades 0-2		Grade 3		Grade 4	
		No.	%	No.	%	No.	%		No.	%	No.	%	No.	%
Hemoglobin														
Grades 0-2	105	27	26	62	59	16	15	61	33	54	19	31	9	15
Grade 3	29	2	7	17	59	10	35	14	3	21	5	36	6	43
Grade 4	9	0	0	3	33	6	67	6	0	0	3	50	3	50
WBCs														
Grades 0-2	117	49	42	47	40	21	18	71	61	86	8	11	2	3
Grade 3	27	0	0	9	33	18	67	15	2	13	11	73	2	13
Grade 4	5	0	0	0	0	5	100	3	0	0	0	0	3	100
Absolute neutrophil count														
Grades 0-2	66	12	18	11	17	43	65	36	27	75	6	17	3	8
Grade 3	22	2	9	0	0	20	91	12	2	17	5	42	5	42
Grade 4	23	2	9	1	4	20	87	9	1	11	0	0	8	89
Lymphocytes														
Grades 0-2	71	23	32	31	44	17	24	38	27	71	9	24	2	5
Grade 3	28	0	0	13	46	15	54	19	2	11	11	58	6	32
Grade 4	9	1	11	2	22	6	67	9	1	11	3	33	5	56
Platelets														
Grades 0-2	80	34	43	19	24	27	34	53	38	72	11	21	4	8
Grade 3	34	0	0	7	21	27	79	18	0	0	4	22	14	78
Grade 4	24	0	0	0	0	24	100	12	0	0	0	0	12	100

NOTE. The No. (n) for each treatment group is the No. of patients with a baseline toxicity grade and at least one postbaseline toxicity grade.
Abbreviation: NCI CTC, National Cancer Institute Common Toxicity Criteria.
*Postbaseline toxicity grade is the maximum grade after baseline and before the end of study.
†Includes all patients exposed to azacitidine, including patients who crossed over to azacitidine from observation.

from grade 0 to 2 at baseline to grade 3 to 4. Azacitidine was associated with worsening of pre-existing cytopenias in ≤ 78% of patients in Protocol 9221. In general, the percentage of patients with shifts from grade 0 to 2 to grade 4 in hematology values was greatest during cycle 1 and then decreased with subsequent cycles.

AEs. The overall rate of AEs per patient-year in the observation group (2.06 patients with events per patient-year of exposure) was nearly twice the rate in the azacitidine group (1.09 patients with events per patient-year of exposure; Table 7). Of the most frequently observed AEs, GI events and injection site reactions occurred at a greater rate in the azacitidine group compared with the observation group. Hematologic events characteristic of MDS generally occurred at a greater rate in the observation group.

Rates of infection and bleeding. To determine whether treatment was associated with an increase in either infection or bleeding, we examined the rates of commonly reported AEs of infection or bleeding. Infections occurred in the observation group at nearly 1 per patient-year, which is similar to the rate previously reported.[9] Treatment with azacitidine did not increase the rate of infection. The rate of infection per patient-year was 0.64 in the azacitidine group and 0.95 in the observation group. Clinically significant infections were similar to the most common sites of infection (lung, urinary tract, and the bloodstream) typically observed in patients with MDS,[9] with no apparent increase in the azacitidine group (Table 8). In the observation group, infection with pneumonia/sepsis was the cause of death in month 3 of one (2%) of the 41 observation patients who did not cross over during the study. Among 150 azacitidine-treated patients, infection (pneumonia, cycle 68; infection, cycle 4; and probable infection, cycle 2) was the cause of death in three patients (2%).

There was no increase in rates of bleeding with azacitidine. The overall rate (patient-years) of bleeding was 0.56 in the azacitidine group and 0.60 in the observation group. Clinically relevant bleeding in the GI, CNS, renal, and pulmonary systems seemed to occur at a similar rate (Table 9). The incidence of bleeding leading to death seemed similar between the observation (2%; one of 41 patients) and azacitidine groups (1%; two of 150 patients). Events leading to death included a subdural hematoma (cycle 5) in the observation group and two episodes of intracranial hemorrhage (cycles 1 and 5) in the azacitidine group.

DISCUSSION

The findings of this study, which are based on more contemporary classification and response criteria, validate the previously published results[1-3] and further our understanding of the activity of azacitidine in the treatment of MDS. Furthermore, these results provide additional findings based on a thorough recollection of data in a manner consistent with Good Clinical Practice, which included a complete safety database, and provide further support for the significant improvement in the quality of life of patients treated with azacitidine reported in a CALGB companion study.[4]

When the IWG response criteria were applied to all three studies, overall response rates were generally consistent among the studies and

Table 7. Most Frequently* Observed Adverse Events (NCI CTC grades 1-4) by Patient-Years of Exposure in Protocol 9221

Adverse Event‡	All Azacitidine Patients† (n = 150)		Observation Patients (n = 92)	
	Patients With Events Per Patient-Year of Exposure§	No. of Patients	Patients With Events Per Patient-Year of Exposure§	No. of Patients
Total exposure, patient-years	138.2		43.2	
At least 1 adverse event	1.09	150	2.06	89
Anemia NOS	0.77	107	1.37	59
Thrombocytopenia	0.74	102	0.97	42
Nausea	0.72	100	0.37	16
Pyrexia	0.56	77	0.65	28
Leukopenia NOS	0.55	76	0.63	27
Vomiting NOS	0.52	72	0.12	5
Fatigue	0.42	58	0.53	23
Constipation	0.42	58	0.14	6
Diarrhea NOS	0.39	54	0.30	13
Neutropenia	0.37	51	0.23	10
Injection site erythema	0.35	49	0	0
Cough	0.34	47	0.32	14
Dyspnea NOS	0.34	47	0.25	11
Ecchymosis	0.32	44	0.32	14
Weakness	0.32	44	0.44	19
Rigors	0.28	39	0.23	10
Injection site pain	0.26	36	0	0
Arthralgia	0.26	36	0.07	3
Headache NOS	0.25	34	0.23	10
Pain in limb	0.25	34	0.12	5
Anorexia	0.23	32	0.14	6
Pharyngitis	0.23	32	0.16	7
Contusion	0.22	31	0.21	9

Abbreviations: NCI CTC, National Cancer Institute Common Toxicity Criteria; NOS, not otherwise specified.
*More than or equal to 20.0% frequency in either treatment group.
†Includes all patients exposed to azacitidine, including patients who crossed over to azacitidine from observation.
‡Multiple reports of the same adverse event term for a patient are only counted once within each treatment group.
§Total exposure for azacitidine is the cumulative time from the first dose to the end of study (30 days after last dose), and for observation, total exposure is the cumulative time from random assignment to withdrawal from study or day before cross over.

with the original CALGB response rates. However, the IWG criteria had lower sensitivity in determining PR rates when compared with the CALGB criteria. IWG criteria had lower sensitivity to discriminate responses, as demonstrated by 17% of patients in the observation-only group who qualified for an IWG response of HI (10% minor hematologic response) compared with only 5% of patients using the original CALGB criteria.

The overall IWG response rates for patients with a retrospective diagnosis of WHO AML were encouraging. Although the CR rate of 9% using IWG MDS response criteria is not outstanding

Table 8. NCI CTC Grades 1 to 4 Infection Rates (patient-years of exposure) in Protocol 9221

Adverse Event†	Azacitidine Patients* (n = 150)		Observation Patients (n = 92)	
	Patients With Events Per Patient-Year of Exposure‡	No. of Patients	Patients With Events Per Patient-Year of Exposure‡	No. of Patients
Total infections§	0.64	89	0.95	41
Candidal infection NOS	0.04	6	0.07	3
Cellulitis	0.09	13	0.09	4
Herpes simplex	0.09	13	0.12	5
Oral candidiasis	0.04	5	0.07	3
Pneumonia NOS	0.10	14	0.12	5
Sepsis NOS	0.04	6	0.12	5
Urinary tract infection NOS	0.09	12	0.12	5

Abbreviations: NCI CTC, National Cancer Institute Common Toxicity Criteria; NOS, not otherwise specified.
*Includes all patients exposed to azacitidine, including patients who crossed over to azacitidine from observation.
†Multiple reports of the same adverse event term for a patient are only counted once within each treatment group.
‡Total exposure for azacitidine is the cumulative time from the first dose to the end of study (30 days after last dose), and for observation, total exposure is the cumulative time from random assignment to withdrawal from study or day before cross over.
§From infections and infestations system organ class using MedDRA, version 5.0 (Northrop Grumman, Los Angeles, CA).

Table 9. NCI CTC Grades 1 to 4 Bleeding Rates (patient-years of exposure) in Protocol 9221

Adverse Event†	Azacitidine Patients* (n = 150)		Observation Patients (n = 92)	
	Patients With Events Per Patient-Year of Exposure‡	No. of Patients	Patients With Events Per Patient-Year of Exposure‡	No. of Patients
Total bleeding§	0.56	77	0.60	26
GI disorders§	0.26	36	0.25	11
GI hemorrhage	0.03	4	0	0
Gingival bleeding	0.13	18	0.09	4
Hemorrhoidal hemorrhage	0.04	6	0	0
Melena	0.03	4	0.05	2
Oral hemorrhage	0.04	5	0.02	1
Rectal hemorrhage	0.05	7	0.05	2
Total nervous system disorders§	0.01	2	0.02	1
Intracranial hemorrhage	0.01	2	0	0
Subdural hematoma	0	0	0.02	1
Total renal and urinary disorders,§ hematuria	0.05	7	0.07	3
Total respiratory, thoracic and mediastinal disorders§	0.22	30	0.23	10
Epistaxis	0.18	25	0.21	9
Hemoptysis	0.05	7	0.02	1

Abbreviations: NCI CTC, National Cancer Institute Common Toxicity Criteria; NOS, not otherwise specified.
*Includes all patients exposed to azacitidine, including patients who crossed over to azacitidine from observation.
†Multiple reports of the same adverse event term for a patient are only counted once within each treatment group.
‡Total exposure for azacitidine is the cumulative time from the first dose to the end of study (30 days after last dose), and for observation, total exposure is the cumulative time from random assignment to withdrawal from study or day before cross over.
§System organ class using MedDRA, version 5.0 (Northrop Grumman, Los Angeles, CA).

relative to standard AML remission induction chemotherapy, the prolongation in survival time to 19.3 months exceeds that typically seen with standard induction chemotherapy, suggesting that azacitidine may alter the natural history of the disease independent of CR response criteria.[10] Furthermore, treatment with azacitidine is associated with significant reduction in risk of transformation to AML and a significant prolongation of survival in patients with high-risk MDS, including RAEB patients, RAEB-T patients $\geq$ 65 years of age, and patients with equivalent intermediate-2 and high-risk disease.[11,12] This finding and the data presented in this article suggest a paradigm shift, with azacitidine altering the natural history of MDS by modulating the behavior of the MDS clone without necessarily eradicating it. In addition, treatment with azacitidine significantly delays the onset of RBC and platelet transfusions in patients who are transfusion independent at study entry. These findings warrant further studies of azacitidine in patients with smoldering AML with multilineage dysplasia (ie, patients who were previously diagnosed as having RAEB-T).

The time to response data indicate that azacitidine can have an effect at the bone marrow level as early as the first treatment cycle. However, for this effect to translate into an improvement in bone marrow function leading to clinically significant increases in peripheral cell counts, the majority of responders can require up to six cycles of azacitidine. To ensure adequate exposure for patients to demonstrate a clinical response, azacitidine should be administered for a minimum of four cycles. Furthermore, patients will most likely continue to require transfusion support during the first several cycles of treatment with azacitidine. In patients who were transfusion dependent at baseline with a response, azacitidine was associated with a median of 9 months of transfusion independence.

Given the underlying disease process and the myelotoxicity of compounds such as azacitidine and decitabine, an increase in rates of infection and bleeding would be expected during treatment. Despite the potential to exacerbate pre-existing cytopenias early in therapy, azacitidine did not increase the rate of infection or bleeding above the rate caused by underlying disease.

This reanalysis demonstrates that azacitidine is effective therapy that directly impacts the disease of MDS rather than just managing the symptoms. It reconfirms the findings discussed in Silverman et al[3] and adds additional data pertaining to safety and more current classification and response criteria. Furthermore, azacitidine warrants additional studies in patients with AML with dysplasia.

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Appendix

The Appendix is included in the full-text version of this article, available online at www.jco.org. It is not included in the PDF version (via Adobe® Reader®).

Authors' Disclosures of Potential Conflicts of Interest

Although all authors completed the disclosure declaration, the following authors or their immediate family members indicated a financial interest. No conflict exists for drugs or devices used in a study if they are not being evaluated as part of the investigation. For a detailed description of the disclosure categories, or for more information about ASCO's conflict of interest policy, please refer to the Author Disclosure Declaration and the Disclosures of Potential Conflicts of Interest section in Information for Contributors.

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