Randomized Controlled Trial of Azacitidine in Patients With the Myelodysplastic Syndrome: A Study of the Cancer and Leukemia Group B

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Purpose: **Patients with high-risk myelodysplastic syndrome (MDS) have high mortality from bone marrow failure or transformation to acute leukemia. Supportive care is standard therapy. We previously reported that azacitidine (Aza C) was active in patients with high-risk MDS.**

Patients and Methods: **A randomized controlled trial was undertaken in 191 patients with MDS to compare Aza C (75 mg/m^2/d subcutaneously for 7 days every 28 days) with supportive care. MDS was defined by French-American-British criteria. New rigorous response criteria were applied. Both arms received transfusions and antibiotics as required. Patients in the supportive care arm whose disease worsened were permitted to cross over to Aza C.**

Results: **Responses occurred in 60% of patients on the Aza C arm (7% complete response, 16% partial response, 37% improved) compared with 5% (improved) receiving supportive care (P < .001). Median time to leukemic transformation or death was 21 months for Aza C versus 13 months for supportive care (P = .007). Transformation to acute myelogenous leukemia occurred as the first event in 15% of patients on the Aza C arm and in 38% receiving supportive care (P = .001). Eliminating the confounding effect of early cross-over to Aza C, a landmark analysis after 6 months showed median survival of an additional 18 months for Aza C and 11 months for supportive care (P = .03). Quality-of-life assessment found significant major advantages in physical function, symptoms, and psychological state for patients initially randomized to Aza C.**

Conclusion: **Aza C treatment results in significantly higher response rates, improved quality of life, reduced risk of leukemic transformation, and improved survival compared with supportive care. Aza C provides a new treatment option that is superior to supportive care for patients with the MDS subtypes and specific entry criteria treated in this study.**

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MYELODYSPLASTIC syndrome (MDS) represents a heterogeneous hematopoietic disorder in which mature blood cells are derived from an abnormal multipotent progenitor cell. The disease is characterized by morphologic features of dyspoiesis, a hyperproliferative bone marrow, and peripheral-blood cytopenias involving one or more lineages.[1] Refractory anemia (RA) with or without ringed sideroblasts can persist for years, but RA with excess blasts (RAEBs) or RAEBs in transformation to leukemia (RAEB-T) exhibit an accelerated course.[2-5] Most patients with high-risk MDS (ie, French-American-British [FAB] subtypes with excess blasts) die within 1 year from progressive bone marrow failure attributable to hemorrhage or infection. In 35% to 40% of patients, transformation to acute leukemia occurs, which is often refractory to present therapy.[1]

Therapies tried for MDS include granulocyte colony-stimulating factor (G-CSF), granulocyte-macrophage colony-stimulating factor (GM-CSF), erythropoietin, and chemotherapy.[6-22] None has altered the natural history of the disease. Supportive care with antibiotics and transfusions is considered the standard of care. Allogeneic bone marrow transplantation, a potentially curative approach, is a realistic option for only approximately 5% of patients.[23-28]

Azacitidine (Aza C), a pyrimidine nucleoside analog, was developed as an antitumor agent.[29-31] In addition to cytotoxic effects, it induces differentiation of malignant cells in vitro.[32-35] Aza C inhibits DNA methyltransferase, the enzyme in mammalian cells responsible for methylating newly

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Table 1. Additional Eligibility Criteria for Bone Marrow Dysfunction in Patients With RA and RARS*

RBC	Symptomatic anemia requiring RBC transfusions for at least 3 months before study entry
Platelets	Thrombocytopenia with two or more platelet counts $\leq 50 \times 10^9$/L or a significant clinical hemorrhage requiring platelet transfusions
WBC	Neutropenia with an ANC $< 1 \times 10^9$/L and an infection requiring intravenous antibiotics

*Patients had to meet at least one of these criteria.

Table 2. Eligibility Criteria

- Age > 15 years
- Life expectancy ≥ 2 months
- Performance status ≤ 2 (NCI scale, 0-4)
- Total bilirubin ≤ 1.5 × ULN
- AST/ALT ≤ 2 × ULN
- Serum creatinine ≤ 1.5 × ULN
- Serum CO_2 ≥ 19 mEq/L
- No previous treatment for MDS with Aza C, G-CSF, GM-CSF, or other hematopoietic cytokines (except for erythropoietin)
- No erythropoietin, corticosteroids, interferon, or retinoids within 1 month before study
- No prior history of leukemia
- No pregnancy on uncontrolled congestive heart failure

Abbreviations: NCI, National Cancer Institute; ULN, upper limit of normal.

synthesized DNA, resulting in synthesis of hypomethylated DNA and changes in gene transcription and expression.[32-34] In patients with beta-thalassemia or sickle-cell anemia, Aza C caused hypomethylation of the gamma globin chain gene with increased production of fetal hemoglobin.[36-38]

The Cancer and Leukemia Group B (CALGB) conducted a phase II study of Aza C administered to 43 hospitalized patients as a continuous intravenous infusion for 7 days every 28 days for 4 months.[39] Responses (complete response [CR], partial response [PR], or improved) occurred in 49% of patients with high-risk MDS (RAEB and RAEB-T). A second phase II study of 67 patients with high-risk MDS showed that Aza C as a subcutaneous daily bolus injection at the same dose and schedule on an ambulatory basis produced comparable results in response rate, response duration, and survival.[40] The present phase III randomized trial compares subcutaneous Aza C treatment with supportive care.

PATIENTS AND METHODS

Patient Selection

All patients fulfilled the FAB classification criteria for MDS.[41-43] Patients with RA or RA with ringed sideroblasts (RARS) met additional criteria of significant marrow dysfunction (Table 1). Bone marrow aspiration and biopsy were required within the 2 weeks before registration. Peripheral-blood films and marrow specimens were independently evaluated through centralized pathology review (D.N.).

Patients with therapy-related MDS were eligible if they were cancer-free for at least 3 years and had not received radiation or chemotherapy for 6 months. Additional eligibility requirements are listed in Table 2. The protocol was approved by the institutional review boards of all participating institutions. Each patient provided written informed consent.

Treatment Regimen

Patients were stratified by FAB subtype and randomly assigned to supportive care or Aza C. The use of all hematopoietic growth factors was prohibited. Transfusions and antibiotics were administered as required. Marrow samples were obtained before study entry, at day 57, and at day 113.

Aza C arm. Aza C (75 mg/m^2/d) was injected subcutaneously in 7-day cycles beginning on days 1, 29, 57, and 85. Aza C, supplied by the National Cancer Institute (Bethesda, MD) in vials of 100 mg of powder plus 100 mg of mannitol, was suspended in 4 mL of sterile water and injected as a slurry with a maximum volume of 4 mL per injection site. If a beneficial effect was not demonstrated by day 57 and no significant toxicity other than nausea or vomiting had occurred, the dose of Aza C was increased by 33%. Once benefit occurred on a particular dosage, Aza C was continued unless toxicity developed. Patients were assessed after the fourth cycle. Those who achieved CR continued on Aza C for three more cycles; those with PR or improvement continued on Aza C until either CR or relapse occurred. Responses were initially evaluated by the treating physician but subsequently were scored independently by two experienced investigators (L.R.S. and R.M.S.) to validate responses. Patients who progressed (see Definitions, below) during the induction phase and those with stable disease at day 113 were classified treatment failures and removed from treatment.

Supportive care arm. After a minimum interval of 4 months of supportive care, patients whose disease was worsening (see Definitions, below) were permitted to cross over to Aza C treatment. Patients could exit supportive care before 4 months but only because of death, withdrawal of consent, transformation to acute leukemia, or a platelet count persistently less than 20×10^9/L after week 8. Patients transforming to acute myelogenous leukemia (AML) exited at any time; those with less than or equal to 40% blasts in the marrow crossed over to Aza C, whereas those with greater than 40% blasts received other treatments.

Cross-over. All data necessary to establish eligibility for cross-over from supportive care to Aza C were independently reviewed by the study chair, whose prior approval was required before cross-over (n = 46 of 49). Cross-over patients were studied and treated identically to patients initially randomized to Aza C.

Quality-of-life assessment. Quality of life (QOL), an integral component of the study, was systematically assessed during standard telephone interviews by one of two trained nurses (E.P.D. or R.O.R.) before randomization and on days 50, 106, and 182. The QOL battery included measures of four dimensions: physical symptoms and functioning, psychological state, social functioning, and sociodemographic characteristics. The questionnaire consisted of two validated scales, the European Organization for Research and Treatment of Cancer (EORTC) QOL and the Mental Health Inventory (MHI). Questionnaires were given or mailed to patients before the telephone interviews; the interview methodologies have been validated in prior CALGB studies.[44]

Table 3. Response Criteria

Trilineage response	≥ 50% restitution of the initial deficit from normal in all three peripheral-blood cell counts and elimination of all blood transfusion requirements		
Monolineage or bilineage response	≥ 50% restitution of the initial deficit from normal in one or two peripheral blood cell counts		
	CR	PR*	Improved†
Bone marrow	M_0 or M_1	≤ 50% of initial bone marrow blasts	—
Peripheral blood			
Counts	H_0	Trilineage response	Monolineage or bilineage response
Blasts	0	0	—
Transfusion	0	0	≤ 50% of baseline
Relapse	> 5% bone marrow blasts or Return to pretreatment blood values or return of RBC or platelet transfusion requirement‡	>30% bone marrow blasts	Return to pretreatment blood values or return of RBC or platelet transfusion requirement§

Abbreviations: M_0, normal bone marrow; M_1, < 5% blasts in the bone marrow, some dyshematopoietic features may persist; H_0, complete normalization of the peripheral-blood counts (ie, hemoglobin ≥ 133 g/L [males], hemoglobin ≥ 117 g/L [females]; WBC ≥ 4.4×10^9/L; ANC 1.8×10^9/L platelets ≥ 140×10^9/L).

*Peripheral-blood criteria alone were used for patients with RA and RARS.

†Criteria for improvement are satisfied by either monolineage or bilineage response or ≥ 50% decrease in transfusion requirement from baseline.

‡For patients with RA or RARS, relapse could be defined on peripheral-blood criteria alone.

§Changes in blood counts secondary to drug-induced myelosuppression did not constitute criteria for relapse.

Definitions

Response criteria are outlined in Table 3.

Relapse of responders. Relapse from CR was defined as greater than 5% myeloblasts in the bone marrow. Relapse from a PR was defined as ≥ 30% myeloblasts in the bone marrow (except for patients with RA and RARS, where peripheral-blood criteria alone or in conjunction with the bone marrow were used). Relapse for improved patients was defined as a decline to pretreatment levels in the blood counts, which were the criteria for response, or the recurrence of a transfusion requirement secondary to disease progression. Reversible changes in blood counts secondary to drug-induced myelosuppression did not constitute criteria for relapse.

Treatment failure in nonresponders. Treatment was considered to have failed in nonresponders receiving supportive care if they advanced to a higher FAB subtype (ie, to RAEB or RAEB-T) or to AML, remained RBC transfusion–dependent before and during study, or developed progressive bone marrow failure, defined as the following: (1) confirmed fall from baseline of greater than 25% in all three peripheral-blood cell lines or greater than 50% fall in two cell lines or a greater than 75% fall in one cell lineage or (2) development of a RBC transfusion requirement (ie, in patients not receiving RBC transfusions before study entry, if the hemoglobin fell to < 9 g/L in patients > 65 years of age or to ≤ 8 g/L if ≤ 65 years of age). Supportive care treatment was also considered to have failed if patients had a platelet count persistently lower than 20×10^9/L after week 8 (N = 9). Nonresponders taking Aza C were evaluated identically for treatment failure, and when treatment failure was present, these patients exited protocol study but were followed for survival.

Statistical Methods

Four analyses, three interim and one final, were planned using O'Brien-Fleming stopping rules. The first three analyses found a significant difference in response between the arms (undisclosed information), but the Data and Safety Monitoring Board recommended continuation of the study so that QOL, survival, and transfusion requirements could be studied in a larger sample. Twenty-six percent of the patient records were independently audited by the CALGB Data Audit Committee for protocol compliance and data quality.

Analyses were performed on an intention-to-treat basis. Patients (n = 20) determined by central pathology review to have acute leukemia at entry were noninformative for AML transformation and the time-to-treatment failure analyses. Response rates of the randomized arms were compared with the χ^2 test of proportions. Survival, time to response, and response duration were estimated with the Kaplan-Meier method and compared with the log-rank test.[45,46] In testing for differences in survival and time to transformation to AML, randomized induction treatments were compared and cross-over was ignored. The two-stage statistical methodology recommended by Gelman et al[47] was used in analyzing the time to AML to account for the competing risk of death.

Prestudy RBC transfusion requirements (present/absent) were calculated. RBC transfusion data were standardized to the number of units per month and the means across time. Differences in transfusion requirements could have been influenced by the loss of patients because of death, cross-over, and dropout (attrition bias) and by physician discretion in the administration of transfusions.

Times to initial response and to best response were measured from study entry to the date that initial and best response criteria were met, respectively. Duration of response was measured from initial response to relapse. Time to treatment failure was measured from study entry to the point of relapse (for responders) or failure (for nonresponders). The time from study entry to transformation to AML or death was chosen as the most meaningful clinical end point, because survival and QOL decline rapidly for patients with MDS after AML develops.

QOL Analysis

A pattern-mixture model was used to examine treatment differences in QOL over time.[48-51]

Table 4. Demographic and Clinical Characteristics at Study Entry

	Aza C		Supportive Care		Total	
	No. of Patients	%	No. of Patients	%	No. of Patients	%
Randomized	99	52	92	48	191	
FAB classification						
RA	17	17	20	22	37	19
RARS	5	5	3	3	8	4
RAEB	32	32	34	37	66	35
RAEB-T	27	27	18	20	45	24
CMMoL	7	7	7	8	14	7
Other*	11	11	10	11	21	11
IPSS risk group†						
Low	2	2	5	6	7	9
Intermediate-1	21	26	16	20	37	45
Intermediate-2	9	11	13	16	22	27
High	7	9	8	10	15	19
Age						
Median	69		67		68	
Range	31-92		35-88		31-92	
Sex						
Male	72	73	60	65	132	69
Female	27	27	32	35	59	31
Prior radiation therapy	8	8	3	1	11	6
Prior chemotherapy	15	15	12	13	27	14
Prior treatment for MDS	16	16	17	18	33	17
Infection requiring treatment	6	6	4	4	10	5
Active bleeding	16	16	18	20	34	18
Patients requiring platelet transfusions‡	18	18	10	11	28	15
Patients requiring RBC transfusions‡	68	69	56	61	124	65
Time from diagnosis to study entry						
Median	77 days		87 days			
Range	1 day-6.4 years		2 days-6 years			

Abbreviation: IPSS, International Prognostic Scoring System.

*Includes 19 AML, one unclassifiable acute leukemia, and one undefined MDS.

†Complete cytogenetic data to determine the IPSS score were only available for 81 patients.

‡During the 3 months preceding study entry.

RESULTS

Patient Characteristics

One hundred ninety-one patients with MDS deemed eligible by treating investigators were entered on CALGB 9221 between February 1994 and May 1996 from 26 main member institutions and their 30 affiliated hospitals. Response and toxicity were analyzed on data available through February 1999. After central pathology review, 20 patients were determined to have AML at study entry. These patients are excluded only from the AML transformation and time to progression analyses. The conclusions were unchanged if these patients were excluded from all analyses (data not shown).

The two arms were evenly balanced at study entry (Table 4). There were no differences by FAB subtype, cytogenetic analysis (n = 81), International Prognostic Scoring System score,[52] or time from diagnosis to study entry. Hematologic parameters at study registration are described in Table 5.

Analysis of Response

Among patients randomized to supportive care, 5% (n = 5) met the criteria for improvement. No patient achieved a CR or PR (Table 6). All five patients categorized as improved either had a rising WBC count or absolute neutrophil count (n = 4) or platelets (n = 1) in the process of transforming from MDS to AML. On the Aza C arm, 60% (n = 60) responded ($P < .0001$), with 7% (n = 7) achieving CR, 16% (n = 16) having PR, and 37% (n = 37) improving. In no case was improvement of cytopenia a component of transformation to AML. The trilineage response was 23% for Aza C and 0% for supportive care. Among the 37 Aza C patients categorized as improved, 35% had increases in all three cell lines (but insufficient to meet criteria for PR), 30% had increases in two cell lines, and 35% had an increase in only a single cell line (Fig 1). Response to Aza C was independent of MDS classification. Responses for patients with RA and RARS (9% CR [n = 2];

Table 5. Hematologic Parameters at Study Entry

	Aza C	Supportive Care	Total
Hemoglobin,* g/L			
Median	90	93	91
Range	53-140	57-140	53-140
WBC, $\times 10^9$/L			
Median	3.6	3.7	3.7
Range	0.7-124.5	0.4-41.2	0.4-124.5
ANC, $\times 10^9$/L			
Median	1.5	1.7	1.6
Range	0.04-90.9	0.1-27.6	0.04-90.0
Platelets, $\times 10^9$/L			
Median	52	72	63
Range	4-479	4-570	4-570

Abbreviation: ANC, absolute neutrophil count.

*Median hemoglobin levels at study entry may reflect transfused values.

18% PR [n = 4]; 32% improved [n = 7]) were comparable with patients with RAEB, RAEB-T, and chronic myelomonocytic leukemia (CMMoL) (8% CR [n = 5], 15% PR [n = 10], 38% improved [n = 25]) among patients classified according to central pathology review (Table 4). Median times to initial response and best response were 64 and 93 days, respectively. The median duration of response among patients who achieved CR, PR, or improvement was 15 months (95% confidence interval [CI], 11 to 20 months) (Fig 2).

Of 49 patients who crossed over from supportive care to Aza C, 47% (n = 23) then responded, with 10% (five patients) achieving CR, 4% (two patients) having PR, and 33% (16 patients) improving. The trilineage response was 14%. Neither age nor sex influenced response rates.

Time to Treatment Failure

With certain exceptions (see above), the study design intended that patients remain on the initial randomization arm for a minimum of 4 months. The median time to exit from supportive care (ie, median time to treatment failure) was 3.8 months (95% CI, 3.5 to 4.0 months; range, 0.6 to > 55 months); the median time to exit from the Aza C arm was 9.1 months (95% CI, 5.6 to 11 months; range, 0.1 to > 44 months) ($P < .0001$).

Analysis of Time-to-AML Transformation or Death

The effects of treatment on transformation to AML or death are illustrated in Fig 3. The median time to event for supportive care was 12 months (95% CI, 8 to 15 months) compared with 21 months (95% CI, 16 to 27 months) for Aza C ($P = .007$). For patients with high-risk FAB subtypes (RAEB, RAEBT, or CMMoL), the median time to AML or death for supportive care was 8 months (95% CI, 4 to 13 months) compared with 19 months (95% CI, 13 to 21 months) for Aza C ($P = .004$). There were an insufficient number of events to estimate medians in the patients with low-risk FAB. Overall, FAB subtype was a significant predictor of time to AML or death ($P = .0003$).

Transformation to AML occurred as the first event in 15% of the patients randomized to Aza C compared with 38% of patients randomized to supportive care ($P = .001$). Indeed, during the first 6 months after study entry, 3% of patients taking Aza C transformed to AML while 24% of patients on supportive care transformed ($P < .0001$). Of the patients who transformed to AML in the supportive care group, 77% were diagnosed at study entry as having RA/RARS or RAEB but not RAEB-T. Figure 4 represents the percent bone marrow myeloblasts at study entry compared with the percentage of blasts in the marrow or peripheral blood (National Cancer Institute criteria) at the time of transformation. To demonstrate the biologic impact on survival of the transformation to leukemia, we performed a landmark analysis after a 12-month date of the association of transformation to AML with survival. The two subgroups included 13 patients who had already transformed to AML by the landmark date and 93 patients who had not yet transformed, both groups independent of therapy. Patients who died before 12 months were excluded. The median additional survival (after the 12-month landmark) was 3 months (95% CI, 1 to 11 months) for patients who had already transformed and 18 months (95% CI, 14 to 26 months) for patients who had not yet transformed to AML ($P < .001$).

Table 6. Analysis of Response

	Aza C		Supportive Care		Cross-Over	
	No. of Patients	%	No. of Patients	%	No. of Patients	%
No. evaluated	99		92		49	
CR	7	7*	0	0	5	10
PR	16	16*	0	0	2	4
Improved	37	37*	5	5	16	33
Total	60	60*	5	5	23	47

*Significant differences between the arms in CR rate ($P = .01$), CR + PR rate ($P < .0001$), and CR + PR + improvement rate ($P < .0001$) were observed.

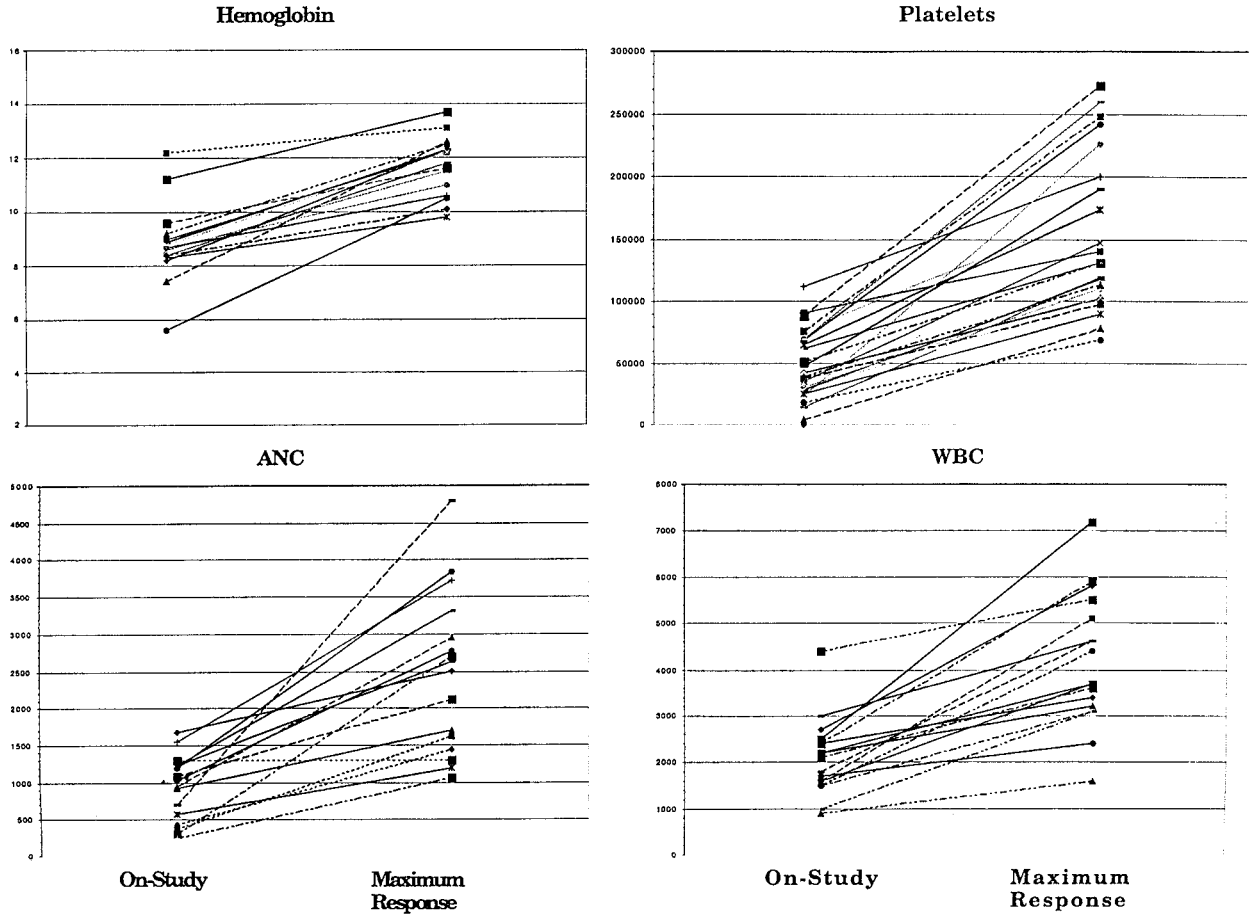

Fig 1. Changes in peripheral-blood counts at the time of response compared with study entry for 37 patients in the Aza C arm classified as improved. Patients who achieved CR or PR are not included.

Effects on RBC and Platelets

The mean number of RBC transfusions increased for the patients taking Aza C in the first month of treatment but thereafter declined, whereas the mean number of transfusions remained stable or increased for patients on supportive care. By definition (Table 3), patients achieving CR or PR had an elimination of RBC or platelet transfusion requirements. Among the 37 patients improved, 73% had an RBC response, 35% (n = 13) had a 50% or greater restitution in the RBC deficit (lineage response), 22% (n = 8) had an elimination of all RBC transfusion requirements, and 16% (n = 6) had a decrease by 50% or greater in RBC transfusions. Thus, among the 99 patients randomized to Aza C, 51% had an RBC lineage response. Among the 65 patients receiving RBC transfusions at study entry, 29 (45%) had an elimination of all transfusions and another six (9%) had a reduction in transfusions by 50%. In addition, lineage responses for platelets and WBC occurred in 47% and 40%, respectively, among those treated with Aza C.

Effects of Treatment on QOL

Patients on the Aza C arm experienced significantly greater improvement over time in fatigue (EORTC, $P = .001$), physical functioning (EORTC, $P = .002$), dyspnea (EORTC, $P = .0014$), psychosocial distress (MHI, $P = .015$), and positive affect (MHI, $P = .0077$) than patients in the supportive care group. Significant differences persisted after controlling for RBC transfusions. Before cross-over, the QOL of patients on supportive care was stable or worsening. After cross-over to Aza C, significant improvements occurred in fatigue (EORTC, $P = .0001$), physical functioning (EORTC, $P = .004$), dyspnea (EORTC, $P = .0002$), and general well-being (MHI, $P = .016$).[50] A complete report of QOL will be presented elsewhere.[51]

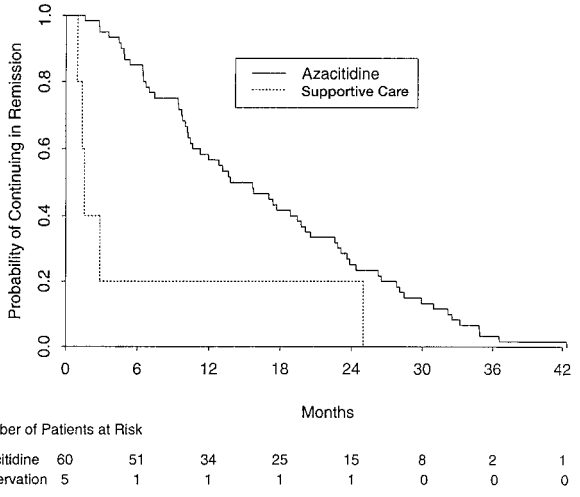

Fig 2. Duration of response. Measured from time of initial response to relapse in patients with CR, PR, or improvement and estimated according to the method of Kaplan-Meier.

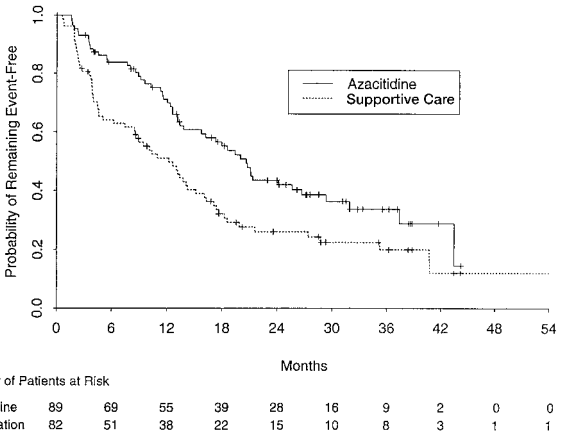

Fig 3. Time to AML transformation or death. Measured from entry on study to the time of first event, either transformation to AML or death, and estimated according to the Kaplan-Meier method.

Detailed analyses make unlikely placebo or Hawthorne[53] effects as explanations for improvements in QOL by Aza C.

Overall Survival

The median survival was 20 months (95% CI, 16 to 26 months) for patients randomized to Aza C compared with 14 months (95% CI, 12 to 14 months) for patients undergoing supportive care (53% of whom received Aza C after cross-over) ($P = .10$)(Fig 5). To eliminate the confounding effect caused by including the 49 cross-over patients in the survival analysis, a landmark analysis was done in which the survival of three subgroups of patients were compared from a 6-month landmark date. These subgroups were supportive care patients who never crossed over or who crossed over only after 6 months, supportive care patients who crossed before 6 months, and patients who were initially randomized to Aza C. The 36 patients who died before the landmark date were excluded. The median survival (after the 6-month landmark date) for these three groups was 11, 14, and 18 months, respectively (Fig 6). The Aza C group was significantly different from the supportive care subgroup who crossed over late or never ($P = 0.03$). Supportive care patients who crossed over early (subgroup 2) had a longer median survival than the patients who crossed over late or never (subgroup 1), although this did not reach statistical significance ($P = .11$). Survival by treatment arm and FAB risk group is demonstrated in Fig 7.

Treatment-Related Toxicity

The most common toxicity of Aza C was myelosuppression. In patients with severe cytopenias from their disease,toxicity was difficult to assess, rendering useless the standard criteria used for hematologic toxicity where the pretreatment marrow is normal. On the basis of standard CALGB criteria, grade 3 or 4 leukopenia occurred in 59%, granulocytopenia in 81%, and thrombocytopenia in 70% of patients receiving Aza C. When hematologic toxicity was reassessed centrally using relative changes in peripheral-blood counts compared with those at study entry, a decrease of 50% to 74% was defined as grade 3 and 75% or greater was defined as grade 4. Based on these criteria, grade 3 or 4 leukopenia occurred in 43%, granulocytopenia in 58%, and thrombocytopenia in 52% of patients receiving Aza C. Toxicity was transient, and patients usually recovered in time for the next treatment cycle. Infection was thought to have been related to treatment in 20% of patients. Nausea or vomiting occurred in 4%. There was one ($\leq$ 1%) treatment-related death.

DISCUSSION

The present results confirm our earlier observations of the beneficial effects of Aza C on bone marrow function in high-risk MDS and extend these findings to symptomatic RA and RARS. The same stringent response criteria used in our original trials of Aza C, developed and defined in the absence of standardized criteria, were used in the present study.[39] The 5% response rate in the supportive care arm indicates that the criteria are sufficiently robust to filter out ordinary variation in blood counts. Incremental changes in peripheral-blood counts among patients improved were sizable (Fig 1). Thus, patients were not categorized as

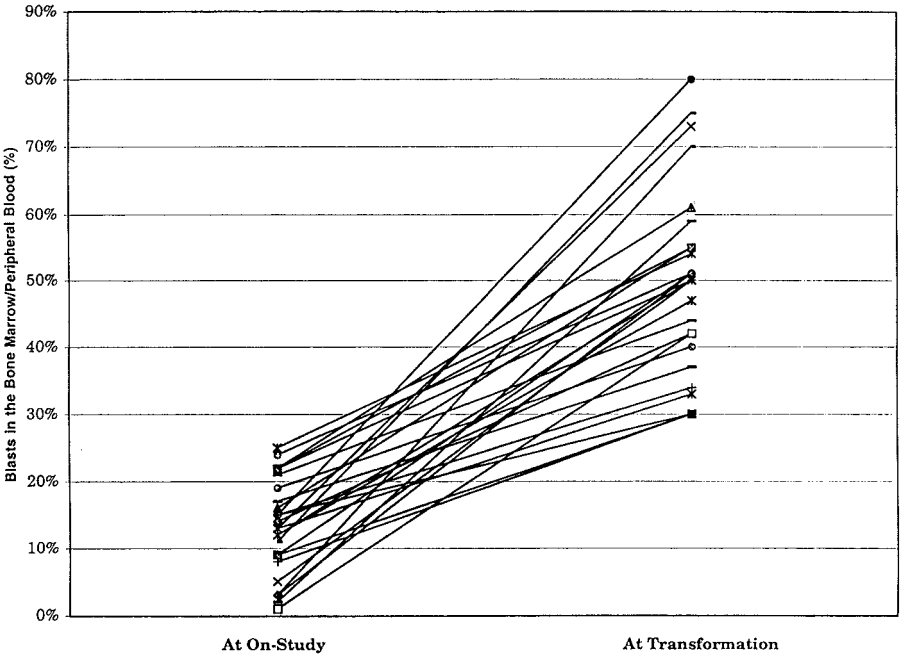

Fig 4. Comparison of myeloblasts in the bone marrow or peripheral blood at study entry and the time of leukemic transformation.

improved on the basis of only a marginal increase in counts as a potential byproduct produced by a quirk of the response criteria. The 60% response rate for Aza C shows that the criteria are sensitive and specific enough to detect biologically important changes, because they are associated with prolonged survival and improved QOL. Our patients were treated at 26 academic centers and 30 of their community affiliates. Thus, our results may predict general medical community achievement.

The number of deaths in the two arms in the first 4 months of study was comparable. The frequency of transformation to leukemia for patients on supportive care was eight-fold higher than patients treated with Aza C in the first 6 months from study entry. Over the entire course of the study, the rate was 2.5-fold higher, the lesser frequency possibly reflecting the fact that many patients were receiving Aza C after cross-over. Differences between the arms in leukemic transformation could not be explained by FAB subtype, International Prognostic Scoring System scores, or time from diagnosis to study entry. Time to leukemic transformation or death represents the purest and most objective manifestation of disease progression for MDS. The landmark analysis demonstrates that transformation to AML has a significantly adverse effect on survival. Aza C delays and decreases transformation to acute leukemia. This is the first description of a drug with this capacity.

The effect of initial treatment with Aza C on overall survival was confounded by the fact that 49 supportive care patients were crossed over to Aza C during their survival follow-up. The landmark analysis diminishes the confounding effect and demonstrates a significant survival advantage in favor of those treated with Aza C initially compared with those not treated or who received treatment only after 6 months of supportive care (Fig 6). A salvage benefit may nonetheless still be important even for patients treated late in the course of their disease.

Significant improvements in QOL, particularly for fatigue, physical functioning, dyspnea, and general well-being, were derived from Aza C treatment, even in the supportive care patients after cross-over. The data indicate that Aza C treatment is more effective in improving QOL than simply raising hemoglobin values with RBC transfusions.

Aza C appears to be superior to other drugs that have been used for MDS. Agents that can induce hematopoietic differentiation in vitro have been extensively tested, and 13-*cis*- and all-*trans*-retinoic acid, 1,25-dihydroxy vitamin D_3, butyrate, cytarabine, and hexamethylene bisacetamide have produced feeble clinical responses. Amifostine has produced responses, but its activity has yet to be fully defined.[54] None of these drugs have caused significant trilineage responses, sustained remissions, or prolonged

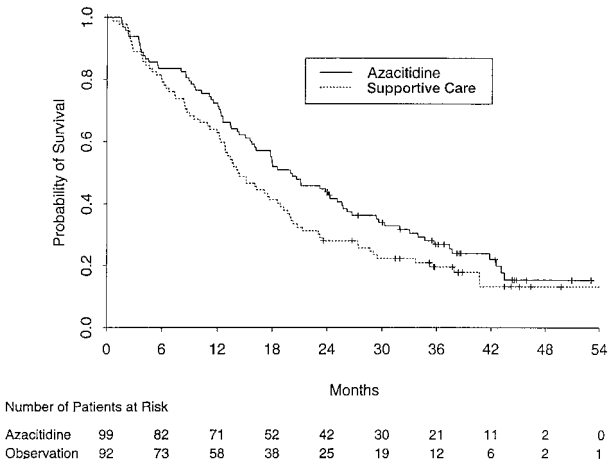

Fig 5. Overall survival by randomized arm and estimated according to the Kaplan-Meier method. Patients who were initially in the supportive care group and crossed over to treatment with azacitidine are included in the supportive care group in this plot.

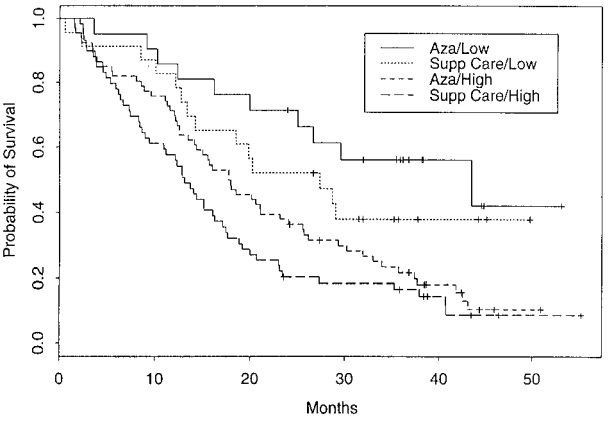

Fig 7. Survival by randomized arm and FAB subtype. FAB subgroups were divided into low-risk (RA/RARS) and high-risk (RAEB, RAEB-T, or CMMoL) groups. Median survival: Aza/Low, 44 months; supportive care (SC)/Low, 27 months; Aza/High, 18 months; SC/High, 13 months.

survival.[55-65] Aggressive antileukemic type therapy and newer agents such as topotecan alone or in combination have produced response rates up to 65% but have not been reported to alter the disease outcome.[66-71]

Four randomized controlled trials have been previously conducted in patients with MDS. *Cis*-retinoic acid demonstrated no advantage compared with placebo.[55,72] Low-dose cytarabine (10 mg/m^2 every12 hours) compared with supportive care led to a 35% hematologic response (using less stringent criteria than the present study) but no differences in time to progression, frequency of transformation to AML, or survival.[22,73] Filgrastim (G-CSF) was compared with supportive care. The death rate for patients with RAEB and RAEB-T was significantly accelerated by G-CSF, with a median survival of 10 months compared with 21 months for supportive care, leading to early termination of the study.[74] Treatment with sargramostim (GM-CSF) resulted in increases in myelomonocytic and lymphoid lineages, with a decrease in frequency of infections in those treated. There were no effects on platelets or red cells. Impact on outcome has not been reported.[11]

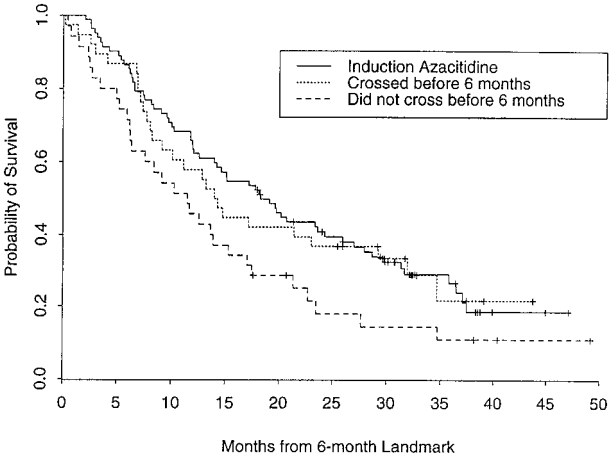

Fig 6. Survival from landmark date by cross-over status (Kaplan-Meier method). Patients were subgrouped as supportive care patients who either never crossed over or crossed over after 6 months, supportive care patients who crossed over before 6 months, and patients who were initially randomized to Aza C.

The mechanism by which Aza C produces its effects is most likely multifactorial. Aza C can produce significant myelosuppression, particularly at higher doses. The doses used in this study and the two prior phase II studies produced marrow hypoplasia in only 10% of patients. Nevertheless, myelosuppression leading to lower peripheral-blood counts and increased RBC transfusion requirements occurred during the first cycle of treatment. Continued treatment without dose reduction led to improved bone marrow function in most patients. Prolonged treatment may have inhibited the MDS clone, permitting residual normal hematopoiesis to emerge. Conversely, Aza C might have exerted a cytotoxic effect on regulatory T cells or other modulatory cells that were inhibiting hematopoiesis.

Aza C could also be acting as a biologic response modifier. The response of hematopoietic progenitors to cytokines is impaired in patients with MDS.[75] This may be attributable in part to abnormalities of the signal transduction pathway downstream from the cytokine receptors.[76-79] In vitro data suggest that Aza C can modulate the cytokine signal transduction pathway, rendering sensitive unrespon-

sive cells to the effects of cytokines, partially restoring normal hematopoietic regulation.[80-82]

As observed in our prior studies, most responding patients demonstrated response beginning in the third or fourth month. This is consistent both with a low-dose cytotoxic effect and with Aza C acting as a biologic response modifier. Incorporation of Aza C into DNA inhibits DNA methyltransferase and induces DNA hypomethylation.[32,83-86] This effect is S-phase dependent, and two or more cycles of DNA synthesis are required to alter gene transcription and expression.[32,84-86] Thus, repetitive exposure on the present low-dose intermittent schedule may have affected small numbers of cells during each treatment, requiring three to four cycles before the effects became clinically apparent. Alteration in the methylation of the *p15* gene has been implicated in transformation of MDS to AML and could be modulated by Aza C, thus reducing risk of leukemic transformation.[87,88]

Although Aza C is active in the present regimen, other doses and schedules might improve its efficacy. Additional studies of Aza C should build on these results. Besides optimization of dose and schedule, combinations with cytokines and other agents that modulate signal transduction are logical areas of exploration. The present study demonstrates that Aza C is effective therapy for patients with MDS with the subgroups and profiles treated in this study. Aza C improves their bone marrow function, decreases and delays significantly transformation to AML, and improves QOL and survival compared with supportive care. These data suggest that Aza C should be considered the treatment of choice for patients with MDS who meet the entry criteria stipulated in this study.

APPENDIX

The appendix listing participating institutions and investigators is available online at www.jco.org.

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