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ORIGINAL REPORT

Phase I Study of Oral Azacitidine in Myelodysplastic Syndromes, Chronic Myelomonocytic Leukemia, and Acute Myeloid Leukemia

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ABSTRACT

Purpose
To determine the maximum-tolerated dose (MTD), safety, pharmacokinetic and pharmacodynamic profiles, and clinical activity of an oral formulation of azacitidine in patients with myelodysplastic syndromes (MDSs), chronic myelomonocytic leukemia (CMML), or acute myeloid leukemia (AML).

Patients and Methods
Patients received 1 cycle of subcutaneous (SC) azacitidine (75 mg/m^2) on the first 7 days of cycle 1, followed by oral azacitidine daily (120 to 600 mg) on the first 7 days of each additional 28-day cycle. Pharmacokinetic and pharmacodynamic profiles were evaluated during cycles 1 and 2. Adverse events and hematologic responses were recorded. Cross-over to SC azacitidine was permitted for nonresponders who received ≥ 6 cycles of oral azacitidine.

Results
Overall, 41 patients received SC and oral azacitidine (MDSs, n = 29; CMML, n = 4; AML, n = 8). Dose-limiting toxicity (grade 3/4 diarrhea) occurred at the 600-mg dose and MTD was 480 mg. Most common grade 3/4 adverse events were diarrhea (12.2%), nausea (7.3%), vomiting (7.3%), febrile neutropenia (19.5%), and fatigue (9.8%). Azacitidine exposure increased with escalating oral doses. Mean relative oral bioavailability ranged from 6.3% to 20%. Oral and SC azacitidine decreased DNA methylation in blood, with maximum effect at day 15 of each cycle. Hematologic responses occurred in patients with MDSs and CMML. Overall response rate (ie, complete remission, hematologic improvement, or RBC or platelet transfusion independence) was 35% in previously treated patients and 73% in previously untreated patients.

Conclusion
Oral azacitidine was bioavailable and demonstrated biologic and clinical activity in patients with MDSs and CMML.

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INTRODUCTION

Azacitidine is a cytidine nucleoside analog with a mechanism of action that involves incorporation into DNA and RNA.[1,2] Data suggest that patients must be exposed to azacitidine over several treatment cycles for optimal therapeutic effect.[3] The requirement for chronic exposure can be explained by drug pharmacokinetics, as azacitidine has a short plasma half-life, and by mechanism of action, as induction of DNA hypomethylation through incorporation into DNA is cell-cycle dependent (S-phase restricted) and DNA remethylation is observed by the end of each treatment cycle.[4]

A treatment regimen facilitating chronic administration may help achieve optimal efficacy outcomes. An oral azacitidine formulation would improve convenience of administration and expand the possibilities of exploring novel maintenance schedules, targeting different malignancies, and testing multiple combinations. A phase 0 trial demonstrated that a single oral azacitidine dose resulted in detectable levels in the blood.[5]

This phase I study sought to identify the maximum-tolerated dose (MTD), dose-limiting toxicities (DLTs), safety, pharmacokinetic and pharmacodynamic profiles, and clinical activity of oral azacitidine in patients with myelodysplastic syndromes (MDSs), chronic myelomonocytic leukemia (CMML), or acute myeloid leukemia (AML).

PATIENTS AND METHODS

The trial was approved by the relevant institutional review boards and ethics committees. All patients gave written informed consent.

Patients

Eligible patients were ≥ 18 years, had an Eastern Cooperative Oncology Group performance status score of 0 to 2, and a diagnosis of MDSs, CMML, or AML according to WHO classification.[6,7] For patients with AML, eligibility was limited to those for whom standard curative measures did not exist or were no longer effective. Exclusion criteria included a diagnosis of acute promyelocytic leukemia, previous treatment with hypomethylating agents within 4 weeks before cycle 1, and anticancer therapy within 21 days before the first dose of study drug, or less than full recovery from any significant toxic effects of prior treatments.

Table 1. Baseline Patient Characteristics (N = 41)

Parameter	No. of Patients	%
Median age, years	70	
Range	31-91	
Sex		
Male	32	78
Female	9	22
MDSs (WHO classification)	29	71
RA/RARS/RCMD	11	27
RAEB-1	12	29
RAEB-2	5	12
MDSs-U	1	2
CMML	4	10
AML	8	20
De novo	4	10
Transformed from MDSs	4	10
IPSS (MDSs patients)*		
Low risk	2	7
Intermediate 1 risk	12	41
Intermediate 2 risk	13	45
High risk	1	3
Not available†	1	3
Hematology		
Median hemoglobin, g/dL	9.3	
Range	6.9-15.1	
Median white blood cell count $\times 10^9$/L	2.4	
Range	0.4-30.2	
Median absolute neutrophil count $\times 10^9$/L	0.8	
Range	0.0-21.7	
Median platelet count $\times 10^9$/L	54.0	
Range	3.0-262.0	
Cytogenetics‡		
Normal chromosomal karyotype	17	49
1 chromosomal abnormality	9	26
2 chromosomal abnormalities	3	9
≥ 3 chromosomal abnormalities	6	17
Prior treatment with hypomethylating agent	16	39
MDSs	13	32
CMML	0	0
AML	3	7

Abbreviations: AML, acute myeloid leukemia; CMML, chronic myelomonocytic leukemia; IPSS, International Prognostic Scoring System; MDSs, myelodysplastic syndromes; MDSs-U, MDSs unclassified; RA, refractory anemia; RAEB, RA with excess blasts; RARS, RA with ringed sideroblasts; RCMD, refractory cytopenias with multilineage dysplasia.

*IPSS score[11] was available for 28 patients with MDSs.

†Patient had a bone marrow transplantation and therefore IPSS risk was not considered applicable.

‡Cytogenetic data were available for 35 patients.

Study Design and Therapy

This open-label, phase I, dose-escalation trial was performed in four participating institutions and evaluated multiple cycles of oral azacitidine administered daily for the first 7 days of a 28-day cycle. The objectives were to determine the MTD, DLTs, and the safety profile of oral azacitidine. Pharmacokinetic and pharmacodynamic profiles of oral and subcutaneous (SC) azacitidine, administered on the same 7-day schedule, were also compared. A secondary objective was to assess the clinical activity of oral azacitidine.

During cycle 1, patients received azacitidine 75 mg/m^2 daily SC for 7 days of a 28-day cycle. During cycle 2 and beyond, patients received oral azacitidine under fasting conditions (ie, no food for 2 hours before and after dosing). The dose of oral azacitidine was escalated following a standard phase I 3 + 3 design. The starting dose was 120 mg and doses were escalated in 60 mg increments up to a dose of 360 mg, followed by 120 mg increments until the MTD was reached. Intrapatient dose escalation was permitted if the dose level to which the patient was escalated was associated with a DLT rate of ≤ 33%. Treatment continued until disease progression, lack of activity, unacceptable toxicity, or patient preference.

The MTD was defined as the highest dose at which no more than 33% of patients experienced a DLT. DLT was defined as: grade ≥ 3 nausea, diarrhea, or vomiting despite adequate/maximal medical intervention; grade ≥ 3 clinically significant nonhematologic toxicity unrelated to underlying disease or intercurrent illness; failure to recover to an absolute neutrophil count (ANC) of higher than 500/μL and/or platelet count of higher than 25,000/μL with hypocellular bone marrow (< 5%) 42 days after starting oral azacitidine (patients with a baseline ANC of ≤ 500/μL and/or platelet count of ≤ 25,000/μL were not evaluable for neutrophil or platelet toxicity); any treatment-related effect resulting in missing ≥ 3 oral azacitidine doses in the 7-day treatment period; or any treatment-related nonhematologic toxicity delaying initiation of the second oral azacitidine cycle by longer than 14 days. Only DLTs that occurred during the first oral azacitidine cycle were considered in determining the MTD. Adverse events were graded using the National Cancer Institute Common Toxicity Criteria for Adverse Events version 3.0.

Table 2. Incidence of Adverse Events According to Severity in ≥ 20% of Patients Treated With Oral Azacitidine (n = 41)

System Organ Class Preferred Term (MeDRA 10.1)	CTCAE Grade 1		2		3		4		Total	
	No.	%	No.	%	No.	%	No.	%	No.	%
Diarrhea	10	24.4	12	29.3	4	9.8	1	2.4	27	65.9
Nausea	8	19.5	10	24.4	3	7.3	0		21	51.2
Constipation	9	22.0	7	17.1	0		0		16	39.0
Vomiting	4	9.8	6	14.6	3	7.3	0		13	31.7
Abdominal pain	6	14.6	4	9.8	0		0		10	24.4
Headache	7	17.1	5	12.2	1	2.4	0		13	31.7
Fatigue	6	14.6	2	4.9	4	9.8	0		12	29.3
Peripheral edema	11	26.8	1	2.4	0		0		12	29.3
Fever	6	14.6	2	4.9	2	4.9	0		10	24.4
Cough	7	17.1	1	2.4	2	4.9	0		10	24.4
Contusion	9	22.0	0		0		0		9	22.0
Dizziness	5	12.2	3	7.3	0		0		8	19.5
Febrile neutropenia	0		0		8	19.5	0		8	19.5

NOTE. This Table includes all adverse events which started during any dosing cycle at which oral azacitidine was administered. Percentages are based on the number of patients who received at least one dose of oral azacitidine. Multiple reports of the same preferred term from a patient are counted only once, using the maximum CTCAE grade.

Abbreviations: CTCAE, National Cancer Institute Common Toxicity Criteria for Adverse Events; MedDRA, Medical Dictionary for Regulatory Activities.

Pharmacokinetic Analysis

Plasma and urine pharmacokinetic evaluation of azacitidine was performed on days 1 and 7 in cycles 1 and 2. Samples were collected up to 8 hours after administration and analyzed using a validated high-performance liquid chromatography/tandem mass spectrometric method. Parameters calculated using noncompartmental method, included maximum observed plasma concentration (C_{max}), time of maximum observed plasma concentration (T_{max}), area under the plasma concentration–time curve from zero to infinity (AUC_{inf}), apparent total clearance (CL/F), relative oral bioavailability (F), and apparent volume of distribution (Vd/F).

Pharmacodynamic Analysis

DNA methylation levels were measured to determine DNA hypomethylating activity of azacitidine when administered SC or orally. Whole blood was collected at baseline and before drug administration on days 3, 8, 15, and 22 of cycle 1, and days 1, 3, 8, 15, 22, and 28 of cycle 2. Genomic DNA was purified from each whole blood sample using the PAXgene Blood DNA System (Qiagen; Valencia, CA). DNA methylation was analyzed using the Infinium Human Methylation27 BeadArray (Illumina; San Diego, CA). In cycle 1, DNA methylation data were generated from blood samples of 15 patients. For 10 of these patients, data were also generated in cycle 2. A methylation ratio, or beta value, for each locus per sample was calculated as methylated signal/(methylated + unmethylated signal). Those with detection $P \leq .05$ were considered high-quality measures. Samples with more than 25,200 high-quality beta values and 26,304 autosomal loci with high-quality beta values in at least half of the samples were used for analyses. The low-quality beta values were reimputed using the pamr.knnimpute function from the R package pamr.[8] Wilcoxon signed-rank tests were performed to identify loci with significant methylation differences at each post-treatment time point versus baseline; $P < .01$ was considered statistically significant. All statistical analyses were carried out in R (R Foundation for Statistical Computing, Vienna, Austria, http://www.R-project.org).

Clinical Activity

Data for clinical activity were evaluated using International Working Group (IWG) 2006 criteria, with modifications as described below, for patients with MDSs or CMML[9] and IWG 2003 criteria for patients with AML.[10] Complete remission (CR), hematologic improvement (HI), and RBC and platelet transfusion independence (TI) were evaluated for patients with MDSs or CMML. Bone marrow CR (mCR) was also evaluated but not included in the overall response rate. RBC transfusion dependence at baseline was defined as ≥ 4 RBC units in the 56 days before cycle 1. Platelet transfusion dependence

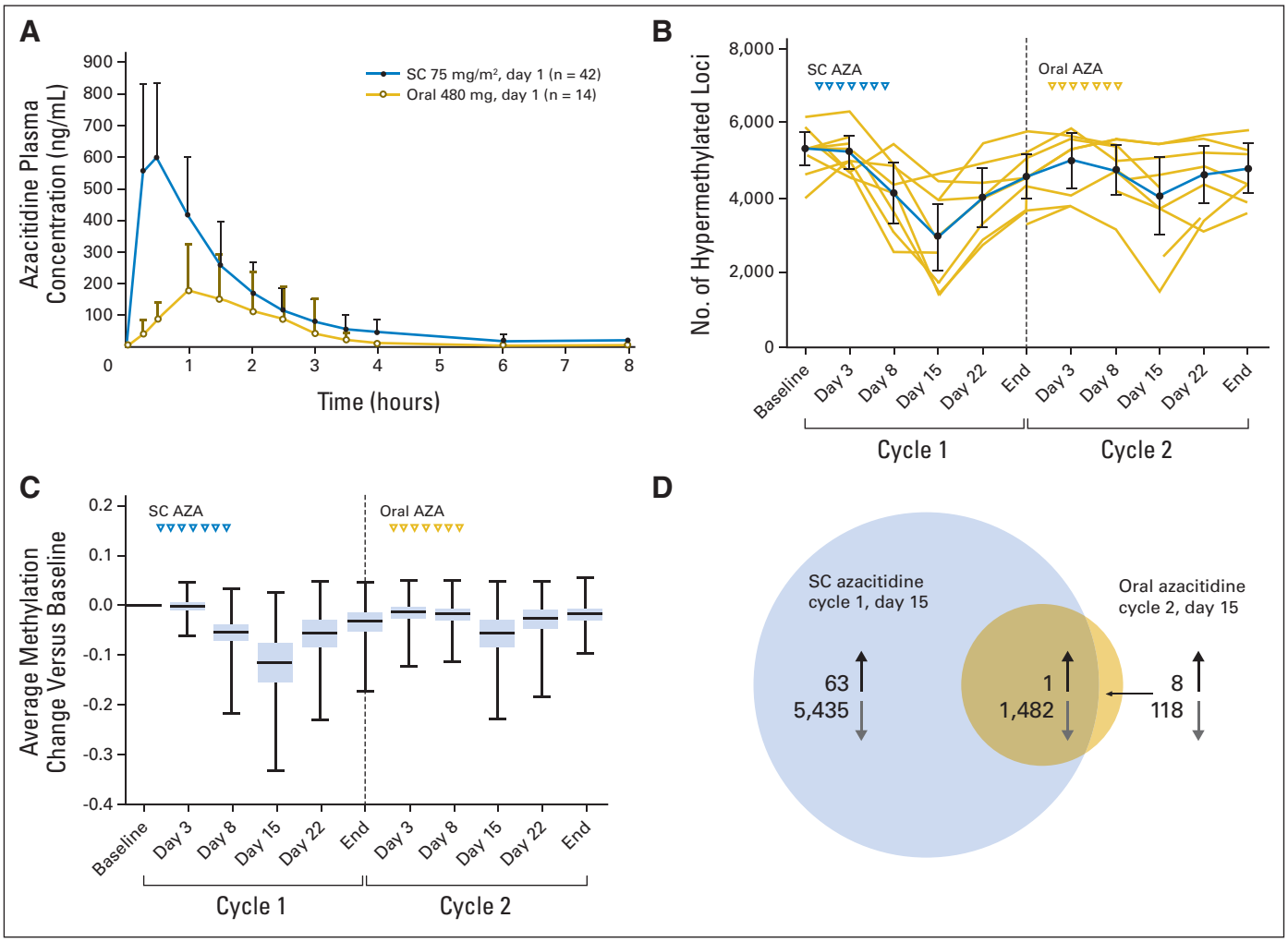

Fig 1. (A) Mean azacitidine (AZA) plasma concentration versus time profiles following single subcutaneous (SC) or oral administration (linear scale). (B) Pharmacodynamics as measured by plotting the numbers of highly methylated loci (beta ≥ 0.7; ± 95% CI) for 10 patients with DNA methylation data in cycles 1 and 2 (gold lines represent individual patients, blue line represents the average). (C) Change in methylation level during treatment with SC or oral AZA for 5,232 loci highly methylated at baseline (blue box represents the 25th to 75th percentile, horizontal band represents the median, vertical line with bars represents minimum and maximum values). (D) Number of significantly differentially methylated loci on day 15 of cycle 1 (SC azacitidine) and on day 15 of cycle 2 (oral azacitidine). Upward arrows denote hypermethylated loci and downward arrows denote hypomethylated loci.

at baseline was defined as ≥ 2 platelet transfusions in the 56 days before cycle 1 (modification to IWG 2006 criteria). RBC and platelet TI were defined as no transfusions in any 56 consecutive-day period on treatment. Patients who achieved ≥ 50% reduction in platelet transfusion requirement, but not platelet TI, in any 56 consecutive-day period on treatment were counted as having achieved HI platelet (HI-P; modification to IWG 2006 criteria). Patients RBC transfusion dependent at baseline achieving a ≥ 50% reduction in RBC transfusion requirement in any 56-consecutive day period and patients not RBC transfusion dependent at baseline, but who achieved a 1.5 g/dL increase in hemoglobin in any 56-consecutive day period on treatment were considered to have achieved HI erythroid (HI-E; modification to IWG 2006 criteria). All patients who received ≥ 1 cycle of oral azacitidine were included in the response analysis. The cutoff date for data in this article was August 19, 2010.

RESULTS

Patient Characteristics

Forty-five patients were treated on a 7-day once-daily schedule. Four patients received the first cycle of SC azacitidine only; three discontinued due to progressive disease (including one death), and one withdrew consent. Baseline characteristics for the remaining 41 patients who received oral azacitidine are presented in Table 1.[11] Cytogenetic data were available at baseline for 35 of 41 patients treated with oral azacitidine; nearly half of the patients had normal karyotype, approximately 25% had a single abnormality, and nearly 20% had a complex karyotype (≥ 3 chromosomal abnormalities). Overall, 16 (39%) of 41 patients had received prior hypomethylating therapy.

Dose Escalation of Oral Azacitidine

No DLTs were observed at dose levels up to 480 mg. DLT was observed at the 600 mg dose, with two (66.7%) of three patients experiencing severe diarrhea, despite adequate medical intervention (grade 3 in one patient and grade 4 in the other). Per protocol, the MTD was exceeded and the previous dose level of 480 mg was determined to be the MTD.

Safety Profile

Table 2 shows the incidence of AEs (any grade) that occurred in ≥ 20% of patients treated with oral azacitidine. The most frequently observed AEs were gastrointestinal disorders, headache, fatigue, and peripheral edema. Other commonly occurring AEs included fever, cough, contusion, dizziness, and febrile neutropenia. Grade 3/4 nausea and grade 3/4 vomiting were each observed in 7% of patients. Grade 3 fatigue was observed in 10% of patients. Diarrhea occurred at grade 3 severity in 10% of patients and grade 4 severity in 2%. Grade 3 febrile neutropenia was observed in eight patients (20%), with four of those having an ANC of ≤ 500/μL at baseline.

Of the 41 patients who received oral azacitidine, 33 terminated from the study as of the date of data analysis, with 17 discontinuing before completing 6 cycles of oral therapy. Reasons for discontinuation included disease progression/treatment failure (n = 10), investigator decision primarily due to absence of observed benefit/response (n = 15), withdrawal of consent (n = 4), AEs (n = 3), and decision to pursue hematopoietic stem-cell transplantation (n = 1). There were three deaths within 28 days of last dose of study drug due to multiple organ failure (n = 1), gastrointestinal hemorrhage (n = 1), and pneumonia plus urinary tract infection (n = 1). No deaths were attributed to study drug. Eight patients remained on the study at the time of data analysis, having each received between 14 and 32 treatment cycles.

Pharmacokinetic Characteristics of Azacitidine

High interpatient variability was noted for all pharmacokinetic parameters. Azacitidine was rapidly absorbed after SC (n = 42) and oral (n = 36) administration, reaching C_{max} within 0.5 hours (range, 0.2 to 1.1 hours) and 1.0 hours (range, 0.3 to 3.6 hours) postdose, respectively. Concentration versus time profiles decreased in a pseudobiphasic manner (Fig 1A). The mean elimination half-life was 1.6 ± 0.7 hours for SC and 0.62 ± 0.25 hours for oral azacitidine. Exposure after single oral administration generally increased with dose (Table 3). For the seven oral dose levels, the mean relative azacitidine oral bioavailability (F) ranged from 6.3% to 20%. The MTD had a mean relative bioavailability of 13% ± 9%. CL/F exceeded hepatic blood flow, indicating extrahepatic metabolism, and Vd/F was greater than total body water, suggesting extensive tissue distribution. The amount of azacitidine recovered in urine relative to dose was small (< 2%) for

Table 3. Day 1 Plasma Pharmacokinetics Parameters After Single Subcutaneous or Oral Azacitidine Administration

Dose	No. of Patients	AUC_{inf} (ng × h/mL) Mean	SD	%CV	CL/F (L/h) Mean	SD	%CV	C_{max} (ng/mL) Mean	SD	%CV	T_{max} (h) Median	Range	Vd/F (L) Mean	SD	%CV	F (%) Mean	SD	Relative Oral Bioavailability
Subcutaneous, 75 mg/m²	42	1,020	440	43*	175	128	73*	650	250	39	0.50	0.2-1.1	410	410	101*	NA		
Oral, mg																		
120	4	62	43	70	4,100	4,860	118	38	24	64	1.48	1.0-2.0	2,930	3,810	130	8.1	5.6	69
180	3	112	64	58	2,330	1,890	81	72	36	50	1.50	1.0-1.5	1,700	1,580	93	6.3	2.3	37
240	3	463	221	48	598	258	43	215	102	47	1.00	1.0-1.5	814	421	52	20.0	9.6	48
300	5	282	88	31	1,180	487	41	144	13	9.2	1.48	1.0-2.0	1,090	626	57	11.5	2.6	23
360	5	311	141	45	1,360	573	42	195	79	40	1.00	0.5-3.6	947	251	27	12.8	2.4	19
480	14	362	253	70	2,140	1,620	76	211	140	66	1.00	0.3-2.5	2,010	1,910	95	12.8	9.4	74†
600	2	502	100	20	1,220	244	20	253	29	12	1.50	1.0-2.0	1,580	1,410	89	14.9	0.8	5

Abbreviations: AUC_{inf}, area under the plasma concentration–time curve from time zero to infinity; CL/F, apparent total clearance; C_{max}, maximum observed plasma concentration; F, relative oral bioavailability; NA, not applicable; T_{max}, time of maximum observed plasma concentration; Vd/F, apparent volume of distribution.
*n = 40.
†n = 13.

SC and oral administration, suggesting that nonrenal elimination is the predominant pathway for clearance. Results after multiple doses were similar to those obtained after a single dose for both administration routes (data not shown). There was no evidence of azacitidine accumulation.

Pharmacodynamics of Azacitidine: Effect on DNA Methylation

DNA methylation was evaluated during cycles 1 and 2 in 10 patients treated with oral azacitidine. The numbers of highly methylated loci were calculated at each time point by averaging across patients the number of loci with methylation ratios ≥ 0.7 (Fig 1B). These numbers decreased after SC and oral administration, with maximal effects at day 15 of each cycle. The reduction in levels of highly methylated loci was not maintained throughout the entire cycle and returned to near-baseline levels by the end of each cycle. SC azacitidine decreased a greater number of loci in comparison to oral azacitidine. The changes in methylation level from baseline across patients for the 5,232 highly methylated loci (average methylation ratio at baseline ≥ 0.7) are represented as box plots (Fig 1C). As with the analysis of total numbers of highly methylated loci, the median DNA methylation of these loci was reduced by 0.115 on day 15 of cycle 1 (SC azacitidine) and 0.055 on day 15 of cycle 2 (oral azacitidine), and returned to baseline levels at the end of each cycle.

Differentially methylated loci at each post-treatment time point compared with baseline were identified in cycles 1 and 2, with the maximum number observed on day 15 of each cycle; 6,981 loci were differentially methylated (6,917 hypomethylated) on day 15 of cycle 1 (SC azacitidine) and 1,609 loci were differentially methylated (1,600 hypomethylated) on day 15 of cycle 2 (oral azacitidine; $P < .01$). In total, 1,482 loci were significantly hypomethylated by both SC and oral azacitidine (Fig 1D), representing 92.6% of all loci significantly hypomethylated by oral azacitidine treatment. These data demonstrate comparable biologic activity with SC and oral azacitidine, albeit to a lesser extent with oral azacitidine.

Clinical Activity of Oral Azacitidine

The median number of oral azacitidine cycles administered to patients with MDSs, CMML, and AML was 6 (range, 1 to 32+), 12.5+ (range, 3 to 28+), and 4.5 (range, 1 to 15), respectively. Treatment duration is summarized in Figure 2. The number of patients from the MDSs, CMML, and AML groups who remained on the study at the

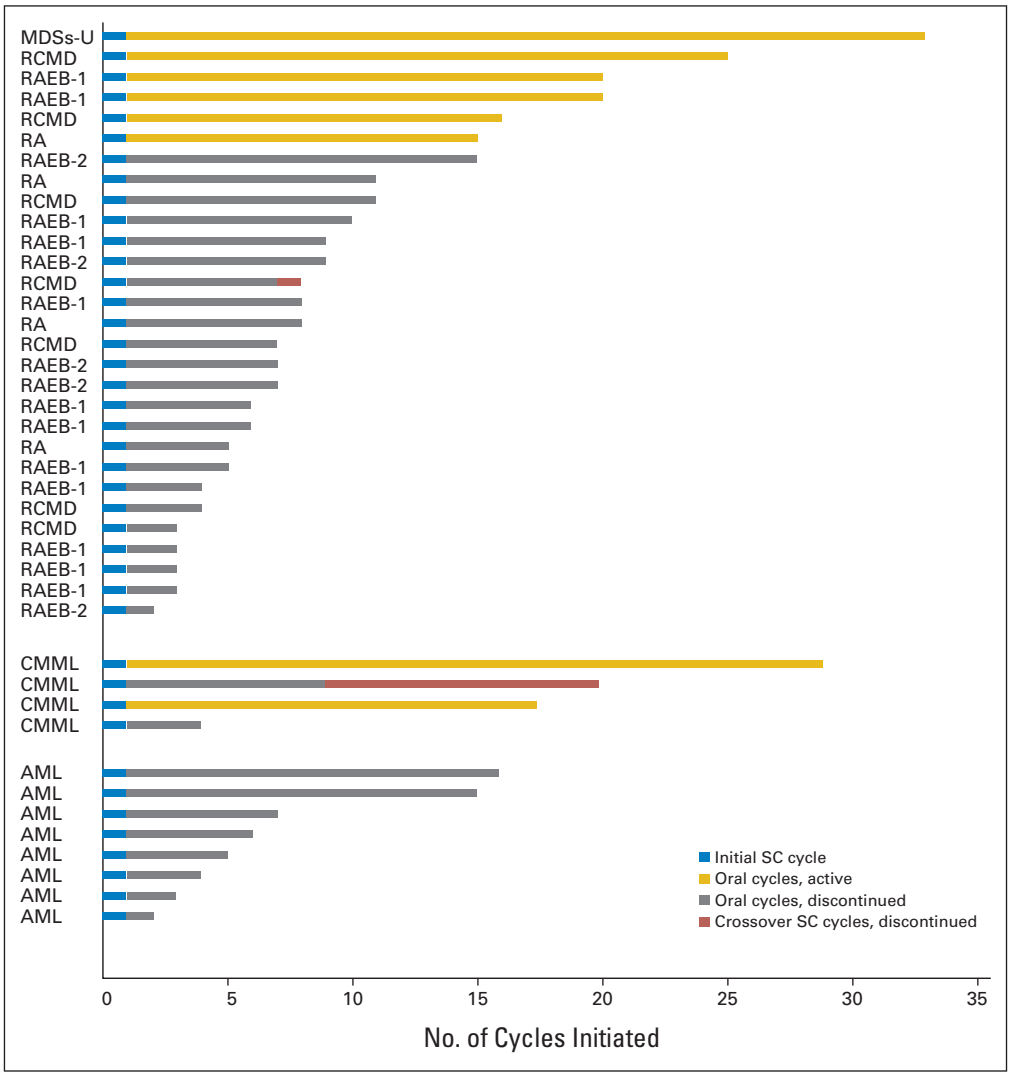

Fig 2. Treatment duration for the 41 patients treated with oral azacitidine (AZA). AML, acute myeloid leukemia; CMML, chronic myelomonocytic leukemia; MDSs-U, myelodysplastic syndromes-unclassified; RA, refractory anemia; RCMD, refractory cytopenias with multilineage dysplasia; RAEB, RA with excess blasts; SC, subcutaneous.

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