REGULAR ARTICLE

A phase 1 study of azacitidine with high-dose cytarabine and mitoxantrone in high-risk acute myeloid leukemia

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Key Points

- The recommended phase 2 dose of AZA is 75 mg/m^2 per day on days 1 to 5 followed by HiDAC/mito once each on days 6 and 10.

- High responses occurred in treatment-naive patients aged ≥60 years with de novo or therapy-related AML and those with *NPM1* or *IDH2* mutations.

In this phase 1 study, azacitidine (AZA) was given before high-dose cytarabine (HiDAC) and mitoxantrone (mito) based on the hypothesis that epigenetic priming with a hypomethylating agent before cytotoxic chemotherapy would improve response rates in patients with high-risk acute myeloid leukemia (AML), including relapsed/refractory disease. The primary objective was to establish the recommended phase 2 dose of AZA given before standard HiDAC/mito. In a dose escalation scheme, 46 patients (median age, 66 years) received AZA at 37.5, 50, or 75 mg/m^2 subcutaneously or IV once daily on days 1 to 5 followed by HiDAC (3000 mg/m^2) and mitoxantrone (30 mg/m^2) once each on days 6 and 10 (the HiDAC/mito dose was reduced 33% in elderly subjects). Two dose-limiting toxicities occurred (both in the same patient): acute liver failure and kidney injury at the 50 mg/m^2 dose. The 30-day induction death rate was 2.2% (1 of 46). The overall response rate, including complete remission and complete remission with incomplete count recovery, was 61% (28 of 46). Previously untreated patients aged ≥60 years with therapy-related AML and de novo AML were more likely to respond than untreated patients with AML progressing from an antecedent hematologic disorder (myelodysplastic syndrome and chronic myelomonocytic leukemia). Patients with favorable European Leukemia Network risk ($P = .008$), *NPM1* mutations ($P = .007$), or *IDH2* mutations ($P = .03$) were more likely to respond, and those with *TP53* mutations ($P = .03$) were less likely to respond. The recommended phase 2 dose of AZA is 75 mg/m^2 per day on days 1 to 5 followed by HiDAC (3000 mg/m^2) and mitoxantrone (30 mg/m^2) once each on days 6 and 10. This trial was registered at www.clinicaltrials.gov as #NCT01839240.

Introduction

Acute myeloid leukemia (AML) has a poor prognosis overall, with a 5-year survival of ~27%.[1] Certain subgroups, including patients with therapy-related AML (t-AML), older patients (ie, those aged ≥60 years), patients with relapsed/refractory disease (RR-AML), and patients with AML progressing from an antecedent hematologic disorder, have a particularly poor outcome.[2-8] In patients with high-risk AML, high-dose cytarabine and mitoxantrone (HiDAC/mito) is an effective and well-tolerated alternative to standard-dose cytarabine with an anthracycline.[9-11]

Dysregulated epigenetic mechanisms, including aberrant DNA methylation, have been implicated in the pathogenesis of AML.[12-14] When used as single agents, the DNA methyltransferase inhibitors azacitidine (AZA) and decitabine have a complete remission (CR) rate between 7% and 25% in treatment-naive patients with AML.[15-17] It is hypothesized that the clinical activity of these agents is mediated at least in part by epigenetic modulation of key genes critical to myeloid leukemogenesis.[18-20] In the current phase 1 study of patients with high-risk AML, including relapsed or refractory disease, AZA was given before HiDAC/mito based on the hypothesis that epigenetic priming with a hypomethylating agent prior to cytotoxic chemotherapy would sensitize malignant cells to chemotherapy and enhance the response to treatment. The primary objective of the current study was to establish the recommended phase 2 dose of AZA when given in combination with HiDAC/mito. Secondary outcomes were to determine the safety of the combination regimen, response rates, and survival.

Materials and methods

Subjects

The study population included patients aged ≥18 years with high-risk AML and an Eastern Cooperative Oncology Group performance status 0 to 2. AML was defined by using the 2008 criteria of the World Health Organization.[21] Patients with high-risk disease were defined as having t-AML, RR-AML, or de novo AML in those aged ≥60 years, AML arising from myelodysplastic syndrome (MDS-AML), myeloproliferative neoplasms in blast phase, and AML arising from chronic myelomonocytic leukemia (CMML-AML). Molecular analysis for NPM1, FLT3, and CEBPA mutations and cytogenetic analysis were performed on pretreatment bone marrow biopsy specimens using Clinical Laboratory Improvement Amendments–approved assays. Genetic risk groups were defined according to the 2010 European Leukemia Network (ELN) risk stratification scheme.[22] Patients were excluded if they had a diagnosis of acute promyelocytic leukemia, major surgery, concurrent anticancer therapy with the exception of hydroxyurea, or participation in other investigational trials within 2 weeks before study entry. There was no limit to the number of previous therapies. Prior AZA or HiDAC exposure was permitted but not in the same schedule as proposed in the current study. This single-center trial was approved by the institutional review board at the University of Chicago and registered at www.clinicaltrials.gov as #NCT01839240. All participants provided written informed consent.

Study design and treatment plan

Cohorts of 3 patients were treated in a 3 + 3 dose escalation scheme. Patients received AZA at 37.5 mg/m², 50 mg/m², or 75 mg/m² subcutaneously or IV once daily on days 1 to 5, followed by cytarabine 3000 mg/m² given IV over 4 hours followed by mitoxantrone 30 mg/m² given IV over 1 hour once each on days 6 and 10. The maximum dose of AZA to be explored was capped at 75 mg/m². Cytarabine and mitoxantrone dose reductions were made for patients aged ≥70 years by 33% to 2000 mg/m² of cytarabine and 20 mg/m² of mitoxantrone. Prophylactic antibiotics and tumor lysis prophylaxis were given according to institutional guidelines.

Table 1. Patient characteristics (n = 46 patients)

Characteristic	Value
Age, median (range), y	66 (21-83)
Sex, n (%)	
Male	27 (59)
Female	19 (41)
Subgroup, n (%)	
Untreated de novo AML	5 (11)
Untreated t-AML	10 (22)
Untreated CMML-AML or MDS-AML*	5 (11)
Relapsed/refractory	26 (56)
ECOG performance status, n (%)	
0	29 (63)
1	15 (33)
2	2 (4)
Risk stratification (ELN criteria), n (%)	
Favorable	8 (17)
Intermediate-1	9 (20)
Intermediate-2	6 (13)
Adverse	23 (50)
Treatment history, n (%)	
First relapse	17 (37)
Beyond first relapse	5 (11)
Primary refractory	4 (9)
Previous hypomethylating agent	14 (30)
All untreated	20 (44)
All untreated aged ≥60 y	17 (37)

ECOG, Eastern Cooperative Oncology Group.
*All patients with previous MDS or CMML received a hypomethylating agent before AML diagnosis.

Toxicity assessment

Dose-limiting toxicity (DLT) was defined as any grade 4 or greater nonhematologic toxicity (except nausea/vomiting lasting ≤48 hours or liver function abnormalities lasting ≤48 hours), grade 3 nonhematologic toxicity lasting ≥7 days, or persistent bone marrow aplasia in the absence of bone marrow involvement with disease for ≥56 days. Cohorts of 3 patients were treated at each dose level, and once the dose had been declared tolerable (0 of 3 DLTs or <2 of 6), additional patients could be enrolled to gain further experience at that dose level while further dose escalation was ongoing. Thus, each dose level had 12 to 18 subjects. All toxicities and adverse events were recorded and graded according to the National Cancer Institute's Common Toxicity Criteria for Adverse Events, version 4.[23]

Efficacy assessment

Bone marrow aspirates and biopsies were performed to evaluate the efficacy of this regimen: a nadir marrow biopsy was performed on day 17, and a biopsy to assess remission status was conducted within 2 weeks of hematologic recovery (defined as absolute neutrophil count ≥1 × 10⁹/L and platelet count ≥100 × 10⁹/L) but no later than day 42. Response criteria for CR, CR with incomplete

Table 2. Toxicity according to worst grade (n = 46 patients)

Toxicity	Grade, n (%)				
	1	2	3	4	5
Supraventricular tachycardia	0 (0)	2 (4)	0 (0)	0 (0)	0 (0)
Heart failure	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)
Pulmonary	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Nausea	16 (35)	1 (2)	0 (0)	0 (0)	0 (0)
Vomiting	5 (11)	0 (0)	0 (0)	0 (0)	0 (0)
Diarrhea	6 (13)	0 (0)	0 (0)	0 (0)	0 (0)
Mucositis	4 (9)	0 (0)	2 (4)	0 (0)	0 (0)
Anorexia	1 (2)	0 (0)	0 (0)	0 (0)	0 (0)
Rash	2 (4)	0 (0)	0 (0)	0 (0)	0 (0)
Headache	2 (4)	0 (0)	0 (0)	0 (0)	0 (0)
Abnormal liver function tests	12 (26)	0 (0)	0 (0)	1 (2)*	0 (0)
Acute kidney injury	2 (4)	1 (2)	0 (0)	1 (2)*	0 (0)
Neutropenic fever	0 (0)	0 (0)	36 (78)	2 (4)	0 (0)
Tongue infection	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)
Pneumonia	0 (0)	0 (0)	8 (17)	0 (0)	0 (0)
Clostridium difficile colitis	0 (0)	0 (0)	3 (7)	0 (0)	0 (0)
Bacteremia	0 (0)	0 (0)	11 (24)	0 (0)	0 (0)
Diverticulitis	0 (0)	0 (0)	1 (2)	0 (0)	0 (0)

*Occurred in the same patient with reported DLT. Note: neutropenic fever was considered a hematologic toxicity.

count recovery (CRi), partial remission, and treatment failure were defined according to the 2010 ELN Working Group recommendations.[22] Overall response rate (ORR) was defined as CR + CRi. Overall survival was defined as time of treatment to time of death. Relapse-free survival was defined as time from treatment to relapse or death in patients who achieved either CR or CRi. Induction death was defined as death from any cause within 30 days of treatment. Patients were removed from the protocol either after completion of all protocol-specific treatments, at patient request or withdrawal of consent, progressive disease, or by principal investigator or treating physician discretion. The data cutoff date was 1 November 2017.

Gene mutation analysis

Pretreatment bone marrow samples were processed by using UCM-OncoPlus, a 1213 gene hybrid capture next-generation sequencing panel.[24] UCM-OncoPlus data were processed by using a custom in-house pipeline, consisting of adapter trimming, alignment to the hg19 version of the human genome, filtering of low mapping quality alignment, and indel realignment. Variant calling was performed after removal of polymerase chain reaction duplicates by using a combination of SAMtools 0.1.19 and UCM-developed software, Variant Inspector. Various filters were applied

Table 3. Number of patients with DLTs

AZA dose	No. of patients enrolled	DLT, n (%) [95% CI]
37.5 mg/m²	18	0 (0) [0-19]
50 mg/m²	16	1 (6.2) [0-30]
75 mg/m²	12	0 (0) [0-27]
Total	46	1 (2.2) [0-12]

to the variant data, including allele frequency of 10% and depth of 50 with Phred quality scores (Q30). These variants were annotated by using Alamut Batch 1.3 software (http://www.interactive-biosoftware.com/) and further filtered based on their 1000 Genomes frequency, coding effect (synonymous variants were removed), and location (only exonic variants with 6 bp at the intronic boundary were considered for this analysis).[25] Commonly observed sequencing artifacts were also removed. Nonsense and frameshift mutations were considered pathogenic. Somatic mutations previously reported in the Catalogue Of Somatic Mutation In Cancer were included as pathogenic mutations.[26]

Statistical analysis

Statistical analysis was performed by using Stata Software. Per the 3 + 3 dose escalation design, the recommended phase 2 dose was defined as the highest dose level such that <2 of 6 patients experienced DLT up to AZA 75 mg/m². Fisher's exact test was performed for analysis of categorical variables. The Kaplan-Meier method was used for survival analysis, and survival curves between groups were compared with the log-rank test. $P < .05$ was considered statistically significant.

Results

Patient characteristics

Between June 2012 and June 2015, a total of 46 patients were enrolled in this phase 1 trial at the University of Chicago Medical Center (Table 1). The median age was 66 years (range, 21-83 years), and 27 (59%) were male. Fifty percent had adverse cytogenetic risk profiles according to the ELN classification.[22] Twenty-six (56%) had relapsed/refractory disease, including 4 of

Table 4. Toxicity according to AZA dose level

| Grade 3 or 4 toxicity | AZA dose, mg/m² per d | | | P |
	37.5 (n = 18)	50 (n = 16)	75 (n = 12)	
Mucositis	1 (6)	0 (0)	1 (8)	.722
	[0-27]	[0-21]	[0-39]	
Abnormal liver function test results	0 (0)	1 (6)	0 (0)	.609
	[0-19]	[0-30]	[0-27]	
Acute kidney injury	0 (0)	1 (6)	0 (0)	.609
	[0-19]	[0-30]	[0-27]	
Neutropenic fever	15 (83)	11 (69)	12 (100)	.099
	[59-96]	[41-89]	[74-100]	
Pneumonia	4 (22)	2 (13)	2 (17)	.884
	[6-48]	[2-38]	[2-48]	
C difficile colitis	0 (0)	3 (19)	0 (0)	.051
	[0-19]	[4-46]	[0-27]	
Bacteremia	2 (11)	4 (25)	5 (42)	.053
	[1-35]	[7-52]	[15-72]	
Tongue infection	1 (6)	0 (0)	0 (0)	1.000
	[0-27]	[0-21]	[0-27]	
Diverticulitis	1 (6)	0 (0)	0 (0)	1.000
	[0-27]	[0-21]	[0-27]	
Mean days to ANC recovery (≥1 × 10⁹/L)	38 ± 8	32 ± 6	29 ± 4	.988
Mean days to platelet recovery (≥100 × 10⁹/L)	31 ± 6	36 ± 8	32 ± 2	.997

Values are n (%) or [95% CI] unless otherwise indicated.
ANC, absolute neutrophil count.

26 with primary refractory disease. Relapsed patients had a median duration of first CR of 11.7 months. The remaining 20 patients (44%) had previously untreated AML, and 17 of these patients were aged ≥60 years. Three previously untreated patients were aged <60 years; 2 of these patients had t-AML, and 1 patient had MDS-AML. All patients with MDS-AML (3 of 3) and CMML-AML (2 of 2) had received previous hypomethylating agent treatment of MDS or CMML.

Safety

During treatment, there were 28 grade 3 to 4 nonhematologic toxicities (Table 2). There were no grade 5 toxicities. Two DLTs occurred (both in the same patient): acute liver failure due to biopsy-proven veno-occlusive disease and acute kidney injury at the 50 mg/m² AZA dose level (Table 3). This patient was a 64-year-old woman with relapsed t-AML who had previous chemo-radiation for both breast cancer and mantle cell lymphoma. Thus, there were 0 of 3, 1 of 6, and 0 of 6 patients with DLTs among the initial cohorts of patients treated at 37.5, 50, and 75 mg/m², respectively, and the recommended phase 2 dose is therefore 75 mg/m². Neutropenic fever was the most common toxicity, with 38 cases (83%), all of which were grade 3 except for 2 cases of grade 4 toxicity. These were not considered DLTs because hematologic toxicity is expected in the course of treatment of AML. One case of grade 4 neutropenic fever occurred in a patient who had neutropenic fever before starting treatment. The patient was a 63-year-old

man who received AZA on protocol at 75 mg/m² but then went off protocol for central nervous system involvement of AML and died of progressive AML. This patient was the only induction death within 30 days (1 of 46 [2%]). The other case of grade 4 neutropenic fever occurred in a 30-year-old man with relapsed AML after previous allogeneic stem cell transplantation who received AZA on protocol at 50 mg/m² followed by HiDAC/mito. He later experienced neutropenic fever and acute respiratory distress syndrome at day 48, possibly due to transfusion-associated lung injury requiring intubation and leading to cardiac arrest. The patient ultimately recovered and was discharged home. No infectious source was identified, and he achieved a CRi. After a follow-up time of 4 years, he remains in a remission. Comparing the various dose levels of AZA, there were no significant differences in toxicities at each dose (Table 4). Overall, mean time of count recovery from initiation of HiDAC was 34.5 days (95% confidence interval [CI], 30.8-38.3) for absolute neutrophil count (≥1 × 10⁹/L) and 32.5 days (95% CI, 29.5-35.5) for platelet count recovery (≥100 × 10⁹/L).

Efficacy

Among all patients in this study, 41% (19 of 46) achieved a CR, with an ORR (CR + CRi) of 61% (28 of 46) (Table 5). There was no difference in response rates between dose levels of AZA (P = .69) (supplemental Table 1). At each dose level of AZA, there was heterogeneity in number of patients according to ELN risk and relapse/refractory status (supplemental Table 2). For untreated patients aged ≥60 years, the ORR was 76% (13 of 17). Among these older untreated patients (supplemental Table 3), patients with t-AML (8 of 8 [100%]) or de novo AML (4 of 5 [80%]) were more likely to respond to treatment compared with patients with CMML-AML and MDS-AML (1 of 4 [25%]) (P = .019). When assessing response according to ELN risk, 8 (100%) of 8 patients with a favorable risk responded to treatment compared with 9 (39%) of 23 for adverse risk (P = .003). Five of the eight favorable risk patients had NPM1 mutations without FLT3-ITD. One patient had t(8;21); one had inv(16); and one had biallelic CEBPA mutations. Patients with previous hypomethylating agent exposure had an ORR (CR + CRi) of 36% (5 of 14) compared with 72% (23 of 32) for those without previous hypomethylating agent exposure (P = .047). For patients with relapsed/refractory disease, those in first relapse had an ORR (CR + CRi) of 71% (12 of 17) compared with 22% (2 of 9) in patients with primary refractory disease or beyond first relapse (P = .038).

The median overall survival for all patients in this study was 10.5 months (range, 0-61+) with a 1-year survival rate of 48% (22 of 46) (supplemental Figure 1). The median relapse-free survival was 11 months (range, 2-61+ months) (supplemental Figure 2). Eighteen of the 46 patients (39%) in this study were able to proceed to allogeneic stem cell transplantation after treatment. Fourteen of the 28 responding patients eventually relapsed. Follow-up time in patients still alive was a median of 40 months (range, 32-61 months). Of the 9 patients remaining alive, 7 received an allogeneic stem cell transplant. At the time of data collection and censoring, patients classified as favorable risk had longer survival, with a median of 45 months compared with 10 months for intermediate-1 risk (P = .023) and 8 months for adverse risk (P = .003).

Thirty-seven genes with pathogenic mutations were identified in this study (supplemental Table 4). Patients with an NPM1

Table 5. Response to treatment

	Response rates				
	CR	CRi	Partial remission	Treatment failure	ORR
Subgroup					
Untreated de novo AML (n = 5)	4 (80)	0 (0)	0 (0)	1 (20)	4 (80)
	[28-100]	[0-52]	[0-52]	[1-72]	[28-100]
Untreated t-AML (n = 10)	5 (50)	3 (30)	0 (0)	2 (20)	8 (80)
	[19-81]	[7-65]	[0-31]	[3-56]	[44-98]
Untreated MDS-AML or CMML-AML* (n = 5)	2 (40)	0 (0)	0 (0)	3 (60)	2 (40)
	[5-85]	[0-52]	[0-52]	[15-95]	[5-85]
Relapsed/refractory AML (n = 26)	8 (31)	6 (23)	0 (0)	12 (46)	14 (54)
	[14-52]	[9-44]	[0-13]	[27-67]	[33-73]
ELN risk					
Favorable (n = 8)	8 (100)	0 (0)	0 (0)	0 (0)	8 (100)
	[63-100]	[0-37]	[0-37]	[0-37]	[63-100]
Intermediate-1 (n = 9)	5 (56)	1 (11)	0 (0)	3 (33)	6 (67)
	[21-86]	[0-48]	[0-34]	[8-70]	[30-93]
Intermediate-2 (n = 6)	3 (50)	2 (33)	0 (0)	1 (17)	5 (83)
	[12-88]	[4-78]	[0-46]	[0-64]	[36-100]
Adverse (n = 23)	3 (13)	6 (26)	0 (0)	14 (61)	9 (39)
	[3-34]	[10-48]	[0-15]	[39-80]	[20-62]
Treatment history					
First relapse (n = 17)	7 (41)	5 (29)	0 (0)	5 (29)	12 (71)
	[18-67]	[10-56]	[0-20]	[10-56]	[44-90]
Beyond first relapse (n = 5)	1 (20)	0 (0)	0 (0)	4 (80)	1 (20)
	[1-72]	[0-52]	[0-52]	[28%-100]	[1-72]
Primary refractory (n = 4)	0 (0)	1 (25)	0 (0)	3 (75)	1 (25)
	[0-60]	[1-81]	[0-60]	[19-99]	[1-81]
Previous hypomethylating agent (n = 14)	4 (29)	1 (7)	0 (0)	9 (64)	5 (36)
	[8-58]	[0-34]	[0-23]	[35-87]	[13-65]
All untreated (n = 20)	11 (55)	3 (15)	0 (0)	6 (30)	14 (70)
	[32-77]	[3-38]	[0-17]	[12-54]	[46-88]
All untreated aged ≥60 y (n = 17)	10 (59)	3 (18)	0 (0)	4 (24)	13 (76)
	[33-82]	[4-44]	[0-20]	[7-50]	[50-93]
Total (N = 46)	19 (41)	9 (20)	0 (0)	18 (39)	28 (61)
	[27-57]	[9-34]	[0-8]	[25-55]	[45-75]

Values are n (%) or [95% CI] unless otherwise indicated.
*All patients with previous MDS or CMML received a hypomethylating agent before the AML diagnosis.

mutation ($P = .007$) or an *IDH2* mutation ($P = .033$) were more likely to respond to treatment, whereas patients with a *TP53* mutation were less likely to respond ($P = .031$) (Figure 1). Five of 6 patients with *IDH2* mutations had R140 mutations, and 3 of these also had concurrent *NPM1* mutations. None of the other genes was associated with response to treatment.

Discussion

This phase 1 trial showed that AZA, at doses up to 75 mg/m^2 per day for 5 days, in sequential combination with HiDAC and mitoxantrone is a safe and effective regimen in patients with high-risk AML. This report is the first of this particular combination, which

resulted in an ORR of 61% with a CR rate of 41%. The 30-day induction death rate was low at 2%. This outcome compares favorably with studies using similar HiDAC/mito regimens for which induction death rates ranged from 9% to 17%.[9-11] Thus, the addition of AZA did not add significant toxicity. Overall, this regimen was well tolerated in this older group of patients with a median age of 66 years. Although we observed no significant difference in efficacy or toxicity between the dose levels of AZA investigated, a dose of 75 mg/m^2 was established as the recommended phase 2 dose due to the absence of DLT, and this was also the maximum dose proposed to be explored. AZA was used in this study at dose levels at which epigenetic modulation is hypothesized to predominate. Therefore, doses beyond 75 mg/m^2 were not
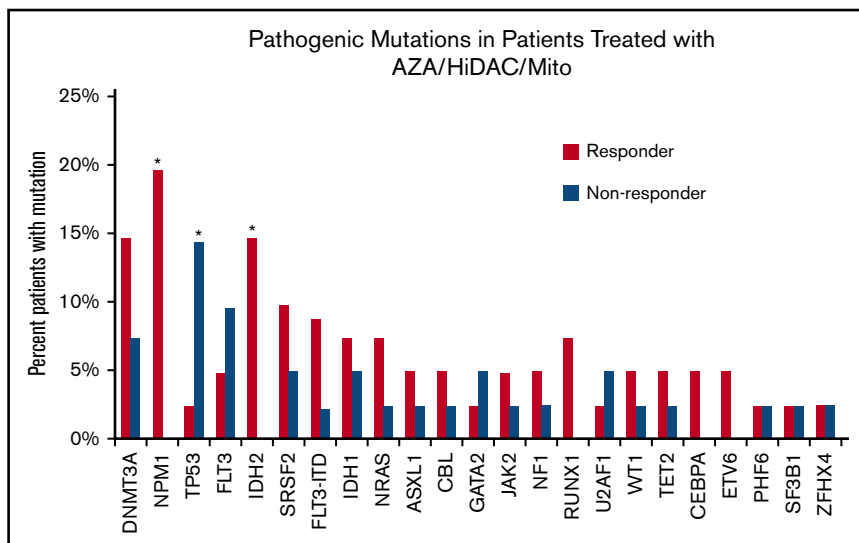
Figure 1. Gene mutations and response to treatment.
Patients had next-generation sequencing performed on pretreatment bone marrow samples, and pathogenic variants were identified. *Genes associated with response, $P < .05$.

explored given the potential for increased cytotoxicity relative to epigenetic effects.

For previously untreated patients aged ≥60 years, the ORR was 77%. Within this group, response rates were particularly high in the t-AML subset (including several patients with adverse risk ELN profiles) and the de novo AML subset. These results are concordant with a previous retrospective study of HiDAC and mitoxantrone on just days 1 and 5 alone conducted at our institution; we found an encouraging ORR (CR + CRi) of 58% (14 of 24) in patients with t-AML and 55% (10 of 18) in patients with de novo AML.[9] The significant activity of the HiDAC/mito regimen in 32 patients with treatment naive t-AML has also been described by our group in a prospective single institution trial; the ORR (CR + CRi) was 66% (21 of 32), and those patients who were aged ≥60 years had an ORR (CR + CRi) of 67% (8 of 12).[10] Nonetheless, the survival for patients with t-AML remains poor, and there are limited prospective treatment data because these patients are often excluded from clinical trials. In 2017, the liposomal formulation of cytarabine and daunorubicin (CPX-351) was approved by the US Food and Drug Administration for adults (ages 60-75 years) with t-AML or AML with myelodysplasia-related changes based on a randomized, multicenter study in which patients with t-AML (n = 30) had an ORR of 47%.[27] Although there were only 8 patients in the current study with t-AML aged ≥60 years, all of these patients responded to treatment with AZA and HiDAC/mito, reinforcing the potential benefit of this strategy for this patient population.

There have been other studies using hypomethylating agents before standard chemotherapy for remission induction in AML. When decitabine was used before standard induction with cytarabine and daunorubicin, a CR rate of 57% (up to 83% after second induction) has been reported without excess toxicity in previously untreated patients aged <60 years.[28] Although both AZA and decitabine are often used interchangeably, we chose to use AZA in the current study because of data suggesting that decitabine and cytarabine share similar mechanisms of resistance.[29] A small phase 2 trial that included 12 untreated patients (age ≥60 years) with AML who received AZA before cytarabine and daunorubicin (7 + 3) resulted in a CR rate of 58% (7 of 12) and an induction death rate of 25%

(3 of 12).[30] In a larger randomized trial including untreated patients (age ≥60 years) with AML, AZA (75 mg/m^2) was given for 5 days before 7 + 3 and resulted in CR rates of 48% compared with 52% in 7 + 3 alone ($P = .58$) and a 30-day mortality of ~6%.[31] Although this earlier randomized study does not support the hypothesis of epigenetic priming with a hypomethylating agent, a different intensive chemotherapy backbone of 7 + 3 was used rather than HiDAC/mito, precluding a direct extrapolation of that experience to the current study. In an unplanned ad hoc analysis comparing the results of this trial of AZA/HiDAC/mito vs a historical cohort of patients with AML treated with HiDAC/mito alone at our institution (supplemental Methods and results; supplemental Table 5), there was a trend in favor of the addition of AZA to HiDAC/mito, and this topic should be explored in a randomized study.

The addition of AZA to HiDAC/mito did not seem to offer an additional advantage to patients with multiply relapsed/refractory disease. Although patients in first relapse had an ORR (CR + CRi) of 71% (12 of 17), the ORR was only 22% (2 of 9) for patients with primary refractory disease or beyond first relapse of disease ($P = .038$). Epigenetic priming with a hypomethylating agent before cytotoxic chemotherapy has been evaluated in this difficult-to-treat population with relapsed/refractory disease and does not seem to be superior to conventional cytarabine-based salvage regimens, although prospective randomized data are lacking.[32]

In the era of next-generation sequencing, there are relatively clear prognostic implications for many of the molecular mutations commonly associated with AML. In this study, patients with *NPM1* mutations were more likely to respond to AZA combined with HiDAC/mito, consistent with our knowledge of mutated *NPM1* being a favorable prognostic marker in AML.[22] Various genes have been analyzed for predicting response to hypomethylating agents, but none has consistently been validated.[33-35] Patients with *IDH2* mutations in this study were also more likely to respond to this regimen. *IDH* mutations have been associated with DNA hypermethylation and implicated in the pathogenesis of myeloid malignancies.[36] Although associated with hypermethylation, *IDH* mutations have not yet been established as predictive biomarkers for response to hypomethylating agents, and results of previous

studies are conflicting.[33,34,37] *IDH2* R140 mutations have strong comutation occurrence with *NPM1* mutations[38]; thus, the response to treatment as observed in this study may simply reflect this association. By contrast, patients with *TP53* mutations were less likely to respond, and this subset represents an ongoing area of unmet need.

This phase 1 study was limited by the small sample size and a heterogeneous population at each dose level of AZA. Despite the trend observed in our study in favor of AZA with HiDAC/mito compared with our historical cohort treated with HiDAC/mito alone, the lack of a randomized comparator and the ad hoc nature of this analysis preclude any definitive conclusions regarding the relative efficacy of our regimen vis-à-vis these historical controls. A randomized study will be required to meaningfully evaluate the contribution of AZA to this combination. In addition, although CRi was included in the ORR in this study, we acknowledge that patients with CRi do not necessarily have the same subsequent prognosis as patients with CR.[39,40] The associations observed here must be confirmed in a larger cohort of patients.

In conclusion, AZA followed by HiDAC and mitoxantrone is a well-tolerated and effective regimen with a low induction death rate. The recommended phase 2 dose of AZA is 75 mg/m^2 per day on days 1 to 5 followed by HiDAC and mitoxantrone (3000 mg/m^2 per day and 30 mg/m^2 per day, respectively, with a 33% dose reduction in elderly subjects) on days 6 and 10. Previously untreated older adults (age ≥60 years) with de novo AML or t-AML should be analyzed in larger randomized studies with this combination. Patient populations with inherently chemoresistant disease such as *TP53* mutated or those with adverse ELN risk are less likely to benefit.

Authorship

Contribution: K.E.C., Y.H.M., and O.O. were responsible for data collection, analysis and interpretation, and manuscript writing; N.J., W.S., and O.O. were responsible for conception and design; T.G.K. performed the statistical analysis; M.G., H.W., and N.F. were responsible for data and sample collection; J.S., S.K., and M.E.M. performed next-generation sequencing/analyzed sequencing data; M.M.L.B. performed the cytogenetic analysis; O.O., L.A.G., A.S.A., H.L., M.J.T., R.A.L., and W.S. contributed patients to the study; and all authors contributed to editing of the manuscript and approved the final version.

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