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Randomized Phase II Trial of Cytosine Arabinoside with and without the CHK1 inhibitor MK-8776 in Relapsed and Refractory Acute Myeloid Leukemia

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Abstract

Purpose—Cytosine arabinoside (AraC) remains the backbone of most treatment regimens for acute myeloid leukemia (AML). Incorporation of AraC into DNA activates checkpoint kinase 1 (Chk1), leading to cell-cycle arrest and diminished AraC cytotoxicity, which can be reversed by the selective Chk1 inhibitor MK-8776. Building on a Phase I trial, we conducted a phase II trial comparing timed sequential AraC with or without MK-8776.

Methods—Patients with relapsed or primary refractory AML were randomized 1:1 to receive either AraC with MK-8776 (Arm A); or AraC alone (Arm B).

Results—32 patients were treated: 14 assigned to Arm A and 18 to Arm B. There were 5 (36%) complete responses (CR/CRi) and 1 (7%) partial response (PR) in Arm A, and 8 (44%) CR/CRis and 1 (6%) PR in Arm B. Median survival did not differ significantly between the two groups (5.9 months in Arm A vs. 4.5 months in Arm B). MK-8776 led to a robust increase in DNA damage in

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circulating leukemic blasts as measured by increased γ -H2AX ($16.9\% \pm 6.1\%$ prior and $36.4\% \pm 6.8\%$ at one hour after MK-8776 infusion, $p=0.016$).

Conclusion—Response rates and survival were similar between the two groups in spite of evidence that MK-8776 augmented DNA damage in circulating leukemic blasts. Better than expected results in the control arm using timed sequential AraC and truncated patient enrollment may have limited the ability to detect clinical benefit from the combination.

Keywords

Leukemia; AML; Chk1; Cytarabine; MK-8776

1. Introduction

Cytosine arabinoside (AraC) is the backbone of both first-line induction and salvage regimens for acute myeloid leukemia (AML).(1) In newly diagnosed AML, AraC-based regimens lead to complete remission (CR) in up to 80% of patients, yet more than half will ultimately relapse.(2) Outcomes of salvage therapy for relapsed and refractory AML patients are worse, with only 23–32% achieving remission with high-dose AraC (HiDAc) alone, and there is scant evidence that adding a second agent improves outcomes.(3–6) The low cure rate among newly diagnosed patients and the limited effectiveness of AraC-based salvage regimens suggest that many patients harbor undetectable residual disease with either *de novo* or acquired AraC resistance. Thus treatments designed to overcome AraC resistance might help to eradicate minimal residual disease (MRD), thereby increasing progression free survival among newly diagnosed patients and improving the rates of remission for relapsed/refractory AML.

AraC exerts its cytotoxic effect on leukemic cells through its incorporation into DNA, which is dependent on the intracellular concentration of and duration of exposure to AraC triphosphate, an active metabolite of AraC.(7) Numerous studies have demonstrated that cellular retention of AraC triphosphate correlates with the likelihood of achieving CR.(8–10) Consistent with this view, changes that reduce AraC triphosphate formation and retention have been reported to cause AraC resistance.(11) Upon incorporation into cellular DNA, AraC activates ataxia telangiectasia and Rad3-related protein (ATR), and checkpoint kinase 1 (Chk1).(12, 13) The ATR-Chk1 checkpoint signaling pathway causes cell cycle arrest and diminishes AraC cytotoxicity by stabilizing stalled replication forks, activating DNA repair, and suppressing apoptosis.(12, 14–18) Indeed, a recent study found that high levels of Chk1 are associated with AraC resistance during induction chemotherapy.(19) Conversely, disruption of Chk1 *in vitro* abrogates these protective effects and yields increased sensitivity to antimetabolites such as AraC.(16, 20–22)

A number of clinical studies have investigated the safety and efficacy of Chk1 inhibition to overcome AraC resistance in AML. In a phase I study, the combination of AraC and tanespimycin, an inhibitor of heat shock protein 90 (Hsp90) that downregulates Chk1 *ex vivo*, led to modest Chk1 downregulation *in vivo* at clinically intolerable tanespimycin doses without any discernible impact on patient outcomes.(23) Similarly, in a trial of AraC with UCN-01, an inhibitor of Chk1 and other kinases, only 1/13 AML patients achieved a CR

despite modest decreases in Chk1 activation.(24, 25) More recently, high content screening identified MK-8776 as a potent and selective Chk1 inhibitor that sensitized AML cells to AraC *in vitro*.(13, 26) Additionally, Chk1 was pulled out as a major hit in an unbiased AML RNAi screen of the human kinome with AraC, validating Chk1 as a high priority target in AML.(27) A phase I study of the AraC/MK-8776 combination in relapsed or refractory acute leukemias established a maximum tolerated dose (2 g/m² of AraC over 72 hours on days 1 and 10 with a 100-mg flat dose of MK-8776 on days 2, 3, 11, and 12) and suggested a possible dose response curve with a response rate of 39% at the highest dose levels.(28) Administration of MK-8776 to patients receiving AraC also led to increased γ -H2AX, an established biomarker of DNA damage,(29–32) in bone marrow blasts from 3 of 5 patients. (28) These observations provided the basis for the current randomized phase II trial of AraC versus AraC plus MK-8776 in relapsed or refractory AML.

The AraC dosing strategy chosen for this study differs from conventional reinduction approaches using HiDAc(3) and is based on the principles of timed sequential therapy (TST). In TST, an initial dose of chemotherapy stimulates humoral factors that increase cell cycling, thereby increasing the incorporation of a subsequent dose of cell-cycle specific agents, such as AraC, into DNA, a key event in cytotoxicity.(33) An *in vivo* model of TST using consecutive cycles of AraC has shown that the timing of the second cycle of AraC is key to extending survival.(34) Based on these findings, TST-based approaches have been used both as initial induction and salvage AML therapy with promising results.(35–37) Thus a salvage regimen based on TST was chosen in combination with MK-8776 for the initial phase I trial.(3, 28)

2. Methods

2.1. Patient eligibility and selection

Between June 2013 and September 2014, patients aged 18–75 years with a pathologically confirmed diagnosis of relapsed or primary refractory AML were enrolled in a multi-institution study with a planned enrollment of 52 patients. Patients were eligible if they received ≥ 2 prior cytotoxic induction regimens and were >2 weeks beyond previous cytotoxic chemotherapy or radiation. The study was conducted in accordance with the Declaration of Helsinki after approval by the ethics committee of each participating center.

2.2. Treatment Schema

Patients were randomized by a centralized computer generated allocation procedure (REDCap) 1:1 to **Arm A:** AraC 2 g/m² over 72 hours intravenous continuous infusion beginning on Day 1 and Day 10 with MK-8776 100 mg over 30 minutes by IV infusion beginning 24 ± 4 hours and 48 ± 4 hours after the start of each AraC infusion on Days 2, 3, 11, and 12 or **Arm B:** AraC alone (Figure 1). Randomization was stratified by risk category: poor risk (primary refractory disease after initial induction, CR1 < 6 months, or 2nd relapse) or intermediate risk (CR1 ≥ 6 months). A treatment cycle was defined as 28 days.

2.3. Response and Toxicity Evaluation

Bone marrow (BM) aspirates and biopsies were obtained before treatment and at hematologic recovery or when leukemia regrowth was suspected. Adverse events were described and graded on the basis of the NCI Common Terminology Criteria for Adverse Events (CTCAE), version 4.0, and treating physician's assessment.

2.4. DNA Damage Assay

BM aspirates were collected from consenting patients in Arm A on day 2 (22 ± 2 hours after the start of AraC infusion) and on day 3 (3 ± 1 hours after the start of MK-8776 infusion). Additional peripheral blood (PB) samples were collected on days 2 and 3 prior to and 1 hour after MK-8776 infusion, and on day 4. A DNA damage assay measuring γ -H2AX in the leukemic blast population was performed on these samples.

2.5. Statistical Analysis

The study was designed to determine if the addition of MK-8776 increased the probability of CR using a Cochrane-Mantel-Haenszel test and interim monitoring for futility. The primary analysis would conclude a significant benefit for the addition of MK-8776 if the one-sided P value from a Cochrane-Mantel-Haenszel test, which tests for an overall treatment difference while adjusting for disease subgroup, was <0.10 . The proposed sample size ($n=52$) yielded an 82% power to detect an increase in the probability of CR/CRi from 25% with AraC alone to 60% with AraC plus MK-8776.

2.6. Please see Supplementary Methods for additional details

3. Results

3.1. Patient Characteristics

Thirty-two patients (Arm A: $n=14$, Arm B: $n=18$) from 4 institutions were randomized, treated, and included in the analysis. An interim analysis for futility after 20 patients showed the predicted probability of rejecting the null hypothesis was 0.27, which did not meet the stopping criteria. The second interim analysis was not performed and accrual was truncated after 32 patients due to the discontinued development of MK-8776.

Clinical demographics and disease biological features of all patients are presented in Table 1. The arms were relatively well balanced in terms of patients with primary refractory disease, in first relapse, and in second relapse. Overall, three patients with protocol-defined intermediate risk disease enrolled in Arm A (21.4%) and eight in Arm B (44.4%). A total of 11 patients (34%) had received a prior BMT (Arm A: 29% vs. Arm B: 39%). Among the ten primary refractory patients, seven received either double induction (3) or a single course of a three-drug induction regimen (cytarabine, an anthracycline, and etoposide) with an escalated dose cytarabine of 667 mg/m^2 via continuous infusion daily for three days (4), while three received only one course of standard dose cytarabine for 7 days and an anthracycline for three days. The groups had similar cytogenetic risk with the majority (59%) of enrolled patients having intermediate risk cytogenetics and the remainder having almost exclusively adverse risk cytogenetics (34%).(38)

3.2. Toxicities

Infectious grade 3/4 treatment-related adverse events were similar in the two groups with a similar percentage of patients suffering febrile neutropenia (Arm A: 64% vs. Arm B: 61%), infectious complications (50% vs. 50%), and sepsis (21% vs. 17%). In terms of other non-hematologic grade 3/4 adverse events, patients in Arm A experienced QT prolongation (21% vs. 0%), hypertension (21% vs. 0%), and GI toxicities (21% vs. 0%); while those in Arm B had increased cardiac (0% in Arm A vs. 17% in Arm B), renal (0% vs. 11%), respiratory (7% vs. 17%), and liver toxicity (7% vs. 17%). All significant grade 3/4 toxicities are presented in Table 2. Two deaths were attributed to treatment, and both were in Arm B.

3.3. Clinical Outcomes

There was no significant difference in response rates between the arms, with 5 patients (36%) in Arm A and 8 patients (44%) in Arm B achieving CR/CRi and 1 patient in each arm achieving a PR (Table 3). The predictive probability of concluding a significant benefit of Arm A over Arm B at the end of the planned study (n=52 patients) based on the response rates from this truncated study was 0.014. While more patients with protocol-defined intermediate risk disease enrolled on Arm B, the odds ratio [95% CI] for response in Arm A over Arm B after adjusting for this stratification factor was 0.84 [0.19, 3.75], p=0.82. A total of 5 patients (Arm A: n=2 and Arm B: n=3) received a second cycle of treatment following a CR (n=3) or PR (n=2).

Subgroups were analyzed based on pre-treatment clinical characteristics including relapsed/refractory status, history of allogeneic bone marrow transplant (BMT), prior treatment with intermediate- or high-dose AraC, and European LeukemiaNet risk status. Logistic regression models testing for interaction between these subgroups and the treatment arms on response failed to demonstrate any significant interactions (Table 3). Importantly, most patients who achieved remission were able to receive a BMT (Arm A: n=2 and Arm B: n=6) or donor lymphocyte infusion (Arm A: n=2 and Arm B: n=1) as consolidation. One-year OS (Figure 2A) did not differ significantly between the arms (Arm A: 36% and Arm B: 28%, stratified log rank P=0.93). Among the responders, one-year EFS (Figure 2B) did not differ significantly between the arms (Arm A: 40% and Arm B: 38%, stratified log rank P=0.90).

3.4. Correlative Studies

DNA damage assessed by staining for γ -H2AX was performed on eight patients in Arm A and demonstrated an increase in γ -H2AX expression on viable circulating blasts from the pre-treatment sample to the sample collected after 24 hours of AraC infusion ($15.5\% \pm 12.5\%$ vs. $8\% \pm 7.7\%$ positive cells, p=0.03, Figure 3). There was a further increase in γ -H2AX expression directly after MK-8776 infusion ($16.9\% \pm 6.1\%$ prior and $36.4\% \pm 6.8\%$ at one hour, p=0.016). However, such an increase was not seen following the second MK-8776 infusion on day 3 ($13.0\% \pm 9.6\%$ prior and $16.0\% \pm 16.2\%$ at one hour, p=0.64). Two patients demonstrated a sustained increase in γ -H2AX among PB blasts 24 hours after the first MK-8776 infusion, and both responded to treatment (1 CR and 1 PR). Interestingly, one patient had a decrease in γ -H2AX staining in PB blasts 24 hours after the first MK-8776 infusion but demonstrated an increase in γ -H2AX staining in BM blasts collected simultaneously; that patient also achieved a CR. All patients who were clinical non-

responders showed decreases in γ -H2AX staining in PB blasts over 24 hours from day 2 to day 3.

Previous studies found that MUS81, MRE11, and RAD50 contributed to increased DNA damage and reduced survival following Chk1 inhibition.(39–41) Consistent with these reports, depletion of these proteins decreased the ability of MK-8776 to sensitize U937 cells to AraC (Figures 4A, 4B), suggesting that levels of MUS81, MRE11 or RAD50 might correlate with responses to AraC plus MK-8776. Immunoblotting of pre-treatment BM blasts for MUS81, MRE11, RAD50, and GAPDH (used as a loading control) showed that levels of all three proteins varied widely among the purified blast populations, but reduced levels of these proteins did not correlate with responses to the drug combination. However, there was a trend towards lower levels of all three in blasts from patients who responded to treatment with AraC including those who also received MK-8776 (Figure 5).

4. Discussion

Increasing the cytotoxicity of AraC and improving responses seen with AraC-based salvage regimens for refractory and relapsed AML remains a challenge. Chk1 inhibition has been shown to augment AraC-induced DNA damage and cytotoxicity in AML *ex vivo*. Accordingly, the ability of the Chk1 inhibitor MK-8776 to improve the response rate or progression-free survival in relapsed/refractory AML was assessed in the present study. Accrual to this randomized study was stopped due to the termination of the clinical development of MK-8776 and its eventual expiration. Interim analysis after 20 patients did not suggest stopping for futility, but the final 32-patient study did not show an improvement in remissions in the AraC/MK-8776 arm. Due to the study's early termination, the arms were not ideally balanced for the stratification factor with a larger proportion of intermediate risk patients in the control arm. This imbalance would be expected to bias the response rate toward the control arm. While we show that the odds ratio for response between the two arms was not significantly different after adjusting for this stratification factor, we cannot exclude the possibility that completion of the trial as planned with balanced enrollment between the two arms may have produced a different outcome.

Although the study is limited by the small sample size, post-hoc subset analyses did not show a clear benefit to the addition of MK-8776 in groups with high cytogenetic risk, prior BMT, or based on relapsed/refractory status. One group of particular interest were those AMLs with adverse and complex cytogenetics, thought to be particularly vulnerable to Chk1 inhibition due to increased levels of Chk1.(42, 43) This AML subset also did not show an improvement in remissions with Chk1 inhibition. The vast majority of patients (85%) in both arms who achieved remission underwent consolidation with a BMT or donor lymphocyte infusion (DLI).

The ability to detect improved outcomes with MK-8776 was limited by the failure to reach the planned accrual and the higher than expected response rate in the control arm. The study design was based on an estimated response rate of 25% in Arm B, but the actual response rate in this arm was 44%. Prior phase III trials in relapsed or refractory AML using HiDac at a dose of 3 g/m² every 12 hours on days 1–6 produced remission rates of 31–32%, but

12% of patients suffered induction death.(3, 5) Thus the cumulative cytarabine dose used in this trial was more comparable to 1 g/m² daily on days 1–5, which produced a response rate (CR+CRi) of 22.9% in 155 relapsed or refractory AML patients but reduced induction death to just 5%.(6) It is possible that AraC given based on TST contributed to the higher than expected response rate in the control group.(3, 4, 44, 45) On the other hand, if the primary benefit of MK-8776 is in eradicating MRD, then this would be reflected in improvements in EFS and OS. Unfortunately, the 1-year EFS and OS were similar in both arms.

Our study did support the tolerability of the MK-8776/AraC combination. This stands in contrast to other agents that are active in the Chk1 pathway, such as tanespimycin and UCN-01, where toxicities have limited their utility in combination with AraC.(23, 24) There was an increase in asymptomatic grade III prolongation of the QTcF interval among patients receiving MK-8776, which was also reported in the phase I trial and is likely attributable to MK-8776.(28) However, this did not translate into increased arrhythmias. More patients in Arm A also developed grade 3 hypertension and gastrointestinal side effects, although the causal link to MK-8776 is unclear. Most notably, there was no increase in AraC-related marrow aplasia, mucosal toxicity or infections related to gastrointestinal barrier breakdown with MK-8776.

Correlative studies to assess DNA damage in leukemic blasts using γ -H2AX were performed on samples from patients in Arm A. Treatment with AraC alone led to an increase in DNA damage in PB blasts in 6/6 patients during the first 24 hours of treatment. The first dose of MK-8776 significantly augmented the AraC-induced DNA damage one hour after dosing. However, this damage was not sustained in PB blasts, as 5/8 patients did not show a further increase of γ -H2AX expression 24 hours later. It is worth noting that the three AMLs with sustained γ -H2AX increases were cases that all achieved a clinical response (2 CRs and 1 PR), whereas none of the patients with only transiently increased DNA damage achieved a response. This is similar to findings in prior studies demonstrating that sustained DNA damage responses correlated with clinical outcomes in AML.(32)

We were intrigued that single agent AraC increased DNA damage in PB blasts in 100% of the patients in this study compared to just 20% of marrow blasts in the phase I trial.(28) This marked difference in DNA damage likely reflects the source of leukemic blasts (PB vs. BM) and suggests a protective effect of the BM microenvironment against AraC-induced DNA damage. In a mouse model of AML, BM stromal cells have been shown to secrete a soluble factor that mediates chemoprotection.(46) Alternatively, marrow stroma-derived SDF-1 has also been implicated in protecting AML marrow blasts from chemotherapy.(47, 48)

In vitro studies suggested the depletion of MRE11, MUS81, and RAD50 decreased the ability of MK-8776 to sensitize cells to AraC and limited the cytotoxicity of Chk1 inhibition.(39–41) Accordingly, we assessed MRE11, MUS81, and RAD50 expression in leukemic blasts prior to treatment to determine their potential as biomarkers for response to Chk1 inhibition and saw a consistent trend towards decreased expression of all three proteins in AMLs that responded to AraC-based treatment. While it is possible that the marrow microenvironment may have played a role in limiting the clinical impact of the combination, MRE11, MUS81, and RAD50 expression may predict AraC responses in poor risk patients.

In summary, this phase II trial of HiDAC with or without MK-8776 in relapsed/refractory AML achieved better than expected results, allowing a substantial fraction of patients to proceed to consolidation with BMT or DLI. The combination was well tolerated but did not improve CR rates or 1-year OS when compared to AraC alone in spite of transiently augmenting DNA damage *in vivo*. However, conclusions about the efficacy of this regimen are hampered by the early termination of this trial. All patients with evidence of sustained increases in DNA damage at 24 hours after MK-8776 infusion responded to treatment. While depletion of the DNA repair enzymes MRE11, MUS81, and RAD50 decreased sensitivity to MK-8776 *in vitro*, low levels of these enzymes in leukemic blasts were not predictive of treatment responses *in vivo*. Determining if the combination of Chk1 inhibition plus AraC can effectively eliminate MRD may prove more fruitful in the upfront setting with less expected inherent drug resistance or in combination with new agents like CPX-351, which is potentiated by Chk1 inhibition *in vitro*. (49, 50) Furthermore the tolerability of MK-8876 may lend itself to rational drug combinations with other targeted agents, such as WEE1 inhibitors. (51)

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- first randomized trial of HiDAc +/- Chk1 inhibition in relapsed/refractory AML.
- response rates and overall survival between the two arms were comparable.
- MK-8776 led to an increase in DNA damage *in vivo* in circulating leukemic blasts.
- Timed sequential therapy led to a 44% response rate in the control arm.

Figure 1.

Treatment Schema.

Patients were randomized 1:1 to the Arm A (AraC 2 g/m² via continuous infusion (CIV) over 72 hours on days 1–3 and 10–12 with MK-8776 100 mg flat dose infused over 30 minutes on days 2, 3, 11, and 12) or Arm B (AraC 2 g/m² via continuous infusion (CIV) over 72 hours on days 1–3 and 10–12).

	N	Median OS [95% CI]	3m OS	6m OS	1y OS	Adjusted HR	95% CI	P
All Patients	32	5.26 [3.38, 17.61]	66 [51, 84]	38 [24, 59]				
Arm A	14	5.91 [4.24, 36+]	71 [51, 99]	43 [23, 78]		1.00	-	
Arm B	18	4.48 [1.64, 36+]	61 [42, 88]	33 [17, 64]	0.69	1.17	(0.53, 2.6)	0.7

Figure 2A

	N	Median EFS [95% CI]	3m EFS	6m EFS	1y EFS	Adjusted HR	95% CI	P
All Patients	13	9.46 [5.03, NA]	85 [67, 100]	54 [33, 89]				
Arm A	5	5.82 [5.03, 26.8+]	80 [52, 100]	40 [14, 100]		1.00	-	
Arm B	8	9.74 [5.68, 26.8+]	88 [67, 100]	62 [37, 100]	0.62	1.39	(0.29, 6.75)	0.68

Figure 2B

Figure 2.

Figure 2A. Overall survival (OS) for the whole cohort and by treatment arm, A or B. OS is defined as the date of study enrollment to date of last follow-up or death. Values for median survival are in months. Other values are survival probability [95% CI].

Figure 2B. Event-free survival (EFS) for the cohort of responders, overall and by treatment arm, A or B. EFS is defined as the date of response to date of relapse, death, or last follow-up. Values for median survival are in months. Other values are survival probability [95% CI].

Figure 3. Percentage of γ -H2AX+ CD34+ cells in the total viable cell population versus collection time for individual patients. Values for individual patients are connected by solid lines

Figure 4.

A. U937 cells were transfected with control luciferase (LUC), MRE11, RAD50, or MRE11 siRNAs. 48 hours later, the cell cultures were divided and immunoblotted for the indicated antigens (A) or were analyzed for viability after treatment with AraC (cytarabine) and MK-8776. B. For the viability assay, cells were plated in triplicate, treated with AraC (cytarabine) at the indicated concentrations, vehicle, or 0.3 μM MK-8776, cultured for 4 days, and subjected to an MTS reduction assay. The figure shows a representative experiment that has been repeated 4 times with similar results

Figure 5.

A. Pre-treatment MUS81 expression from purified patient blast cells. Results are separated by treatment arm and treatment response. The horizontal bar represents the median for each subgroup. B. Pretreatment RAD50 expression from purified patient blasts. Results are separated by treatment arm and treatment response. The horizontal bar represents the median for each subgroup. C. Pre-treatment MRE11 expression from purified patient blast cells. Results are separated by treatment arm and treatment response. The horizontal bar represents the median for each subgroup.

Table 1

Patient Characteristics

Patient Characteristics	All (n=32)	Arm A (n=14) N (%)	Arm B (n=18) N (%)
Median Age (N, range)	60 (29–72)	60 (37–72)	60 (29–71)
Male	17 (53%)	8 (57%)	9 (50%)
Hispanic/Latino	3 (9%)	1 (7%)	2 (11%)
White	22 (69%)	8 (57%)	14 (78%)
Black or African American	8 (25%)	5 (36%)	3 (17%)
Native Hawaiian or Other Pacific Islander	1 (3%)	0 (0%)	1 (6%)
Unknown	1 (3%)	1 (7%)	0 (0%)
Median WBC at entry (n=23)	3.66 K/mm ³	3.76 K/mm ³	3.275 K/mm ³
Primary Refractory	10 (31%)	5 (36%)	5 (28%)
1 st Relapse	15 (47%)	5 (36%)	10 (56%)
CR1 < 6 months	4 (13%)	2 (14%)	2 (11%)
CR1 ≥ 6 months	11 (34%)	3 (21%)	8 (44%)
2 nd Relapse	7 (22%)	4 (29%)	3 (17%)
Prior BMT	11 (34%)	4 (29%)	7 (39%)
Prior CIV or intermittent intermediate- or high-dose AraC ¹	25 (78%)	10 (71%)	15 (83%)
Prior standard-dose AraC ¹	6 (19%)	3 (21%)	3 (17%)
No prior AraC	1 (3%)	1 (7%)	
Site			
Johns Hopkins	23	9	14
BMT of GA	4	2	2
Mayo Clinic- AZ	4	2	2
Mayo Clinic- MN	1	1	0
Cytogenetic Risk ²			
Relapsed Core-Binding Factor (CBF)	1 (3%)	1 (7%)	0 (0%)
Intermediate 1	11 (34%)	4 (29%)	7 (39%)
Intermediate 2	8 (25%)	5 (36%)	3 (17%)
Adverse	11 (34%)	4 (29%)	7 (39%)
Unknown	1 (3%)	0 (0%)	1 (6%)

¹ Standard-dose AraC defined as 100 or 200 mg/m² daily. Intermediate or high-dose AraC defined as 2500 mg/m² daily.

² Dohner H, Estey EH, Amadori S, et al. Diagnosis and management of acute myeloid leukemia in adults: Recommendations from an international expert panel, on behalf of the European LeukemiaNet. *Blood*. 2010;115(3):453–474.

Grade III-IV Toxicities. P-values for Fisher's exact test for differences in the proportion of toxicities between treatment arms. Values shown are the number of patients with the toxicity (percent of patients) [95% exact binomial confidence interval for percent].

Table 2

	All		Arm A		Arm B		P
	N = 32	95% CI	N = 14	95% CI	N = 18	95% CI	
Febrile Neutropenia - no. (%)	20 (62.5%)	[0.44, 0.79]	9 (64.3)	[0.35, 0.87]	11 (61.1)	[0.36, 0.83]	>0.99
Sepsis - no. (%)	6 (18.8)	[0.07, 0.36]	3 (21.4)	[0.05, 0.51]	3 (16.7)	[0.04, 0.41]	>0.99
Infection - no. (%)	16 (50)	[0.32, 0.68]	7 (50)	[0.23, 0.77]	9 (50)	[0.26, 0.74]	>0.99
QT Prolongation - no. (%)	3 (9.4)	[0.02, 0.25]	3 (21.4)	[0.05, 0.51]	0 (0)	[0, 0.19]	0.073
Hypertension - no. (%)	3 (9.4)	[0.02, 0.25]	3 (21.4)	[0.05, 0.51]	0 (0)	[0, 0.19]	0.073
Cardiac Toxicity - no. (%)	3 (9.4)	[0.02, 0.25]	0 (0)	[0, 0.23]	3 (16.7)	[0.04, 0.41]	0.238
GI Toxicity - no. (%)	3 (9.4)	[0.02, 0.25]	3 (21.4)	[0.05, 0.51]	0 (0)	[0, 0.19]	0.073
Renal Toxicity - no. (%)	2 (6.2)	[0.01, 0.21]	0 (0)	[0, 0.23]	2 (11.1)	[0.01, 0.35]	0.492
TLS - no. (%)	1 (3.1)	[0, 0.16]	0 (0)	[0, 0.23]	1 (5.6)	[0, 0.27]	>0.99
Liver Toxicity - no. (%)	4 (12.5)	[0.04, 0.29]	1 (7.1)	[0, 0.34]	3 (16.7)	[0.04, 0.41]	0.613
Respiratory - no. (%)	4 (12.5)	[0.04, 0.29]	1 (7.1)	[0, 0.34]	3 (16.7)	[0.04, 0.41]	0.613
Electrolyte Abnormalities - no. (%)	4 (12.5)	[0.19, 0.53]	5 (35.7)	[0.13, 0.65]	6 (33.3)	[0.13, 0.59]	>0.99

Table 3

CR rates by patient subgroup. P-values from logistic regression models for the interaction between subgroup and treatment arm on response.

	Arm A: CR	Arm A: NR	Arm B: CR	Arm B: NR	OR	Interaction P
All Patients	5	9	8	10	1.42	0.72
Primary Refractory	1	3	3	2	3.76	0.55
First Relapse	3	3	4	6	0.68	
Second Relapse	1	3	1	2	1.41	
Prior BMT	2	2	2	5	0.44	0.22
No Prior BMT	3	7	6	5	2.66	
Prior intermediate- or high-dose ara- C	3	7	6	9	1.53	0.89
No prior intermediate- or high-dose ara-C	2	2	2	1	1.81	
Favorable risk	1	0	0	0		0.96
Intermediate 1 risk	2	2	4	3	1.3	
Intermediate 2 risk	1	4	1	2	1.83	
Adverse risk	1	3	3	4	2.09	