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ORIGINAL ARTICLE

Low Dose Cytosine Arabinoside and Azacitidine Combination in Elderly Patients with Acute Myeloid Leukemia and Refractory Anemia with Excess Blasts (MDS-RAEB2)

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Abstract Only one-third of elderly (>60 years) AML and MDS-RAEB2 patients may receive intensive chemotherapy treatment alternatives that are limited in this patient group due to the potential of severe toxicity. Previous studies have shown that azacitidine and low dose cytarabine treatments may be a beneficial treatment option for these patients. In this study, we aimed to good results with low toxicity in elderly patients. We retrospectively analyzed the AML and MDS-RAEB2 patients who received azacitidine monotherapy and azacitidine and LDL-ara-c combination therapy for a comparison of their response to therapy, survival rates, and toxicity rates and for determining the factors that could affect their overall survival. A total of 27 patients who were diagnosed with de novo AML and MDS-RAEB2 and who received at least four cycles of chemotherapy were included in the study, and the data were evaluated retrospectively. When monotherapy and combination therapy groups were compared, the pretreatment bone marrow blast count was observed to be greater in the combination therapy group. A statistically significant difference was not detected between the groups regarding the response to therapy ratios ($p = 0.161$) (42.9 and 57.1 %, respectively). No difference was detected between the groups regarding therapy-related toxicity. Infections were the most common complication. Progression-free survival was 30.3 % for the azacitidine monotherapy group and 66.7 % for the combination (azacitidine + LD-ara-c) group. The factors influencing the overall survival rate were determined based on the response to the first-line therapies, more than a grade 2 infection, fever, and relapse in a multi-variance analysis. The combination therapy may be a well-tolerated treatment option for the elderly, vulnerable AML patients whose blast count is high in response to therapy rates, overall survival rates, and toxicities are not different, although the pre-treatment bone marrow blast count was greater in the combination therapy groups compared with the monotherapy group.

Keywords Acute myeloid leukemia · Elderly · Azacitidine · Low dose cytarabine

Introduction

Acute myeloid leukemia (AML) is an agressive disease with a poor prognosis. Only 1/3 of the elderly (>60 years) AML patients may receive intensive chemotherapy protocols [1]. The patients who cannot receive intensive chemotherapy are given either only a effective supportive therapy or protocols that contain low doses of cytosine arabinoside (LD-ara-c) [1], azacitidine [2], clofarabine [3], and gemtuzumab ozogamicin [4]. DNA hypermethylation is the most common permanent pathogenetic process observed in MDS and AML patients. DNA methylation may be effectively achieved by the inhibition of DNA methyltransferase [5]. Phase III trials conducted with azacitidine, a DNA methyltransferase inhibitor, showed that azacitidine improved cytopenias and prolonged overall survival compared with beneficial supportive therapies or conventional regimens in MDS and AML patients [2, 6, 7].

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Cytosine arabinoside is the nucleoside analogue of deoxycitidine. LD-ara-c is widely used in the elderly AML patients [5]. The response to cytarabine therapy was shown to be better in the elderly AML patients in the study which was comparing low dose cytarabine and hydroxyurea [1]. In a study conducted on relapsed and refractory high risk MDS and AML patients, the combination of azacitidine and varying doses of LD-ara-c was shown to have a limited effect and was recommended as an alternative therapy [5]. A similar study was conducted on MDS RAEB patients, and it was shown that the response rates increased; however, this combination did not have an effect on leukemic transformation [8].

Based on these studies, we aimed to get good response with low toxicity. We administered to our patients azacitidine plus LD-ara-c combination and thereafter we compared with monotherapy azacitidine which taken patients who were diagnosed as MDS-RAEB2 and AML. We retrospectively analyzed the monotherapy and combination therapy for a comparison of their efficacy and the toxicity rates.

Patients and Study Method

Patients and Data Collection

A total of 32 newly diagnosed AML and MDS-RAEB2 patients who were followed up with in the Hematology Department of Başkent University between December of 2010 and January of 2014 were screened retrospectively. Of these patients, 5 were excluded because they died following one cycle of chemotherapy. Twenty-seven newly diagnosed AML and RAEB2 patients were included in the study. Inclusion criteria included being above 60 years of age, being a newly diagnosed and no history of prior hematological disease and received chemotherapy for AML or MDS-RAEB2 (according to the World Health Organization (WHO)-2008 classification [8], at least four course of azacitidine containing chemotherapy regimen, an agreement to received chemotherapy patients didn't accept to receive standart chemotherapy protocols, patients who have been more than 2 comorbidities with high risk for standart chemotherapy protocol. Exclusion criteria are; AML patients could received the standart chemotherapy did not participate the study, patients received less than four cycle azacitidine containing regimen.

Treatment Method

Azacitidine monotherapy was given to the patients which were diagnosed as MDS-RAEB-2 and have been several comorbidities. LD-ara-c plus azacitidine combination therapy was applied to the patient who were diagnosed AML with less than 2 comorbidities and patients can be tolerated to the combination therapy. Azacitidine monotherapy was applied in the dose of 75 mg/m^2 daily via a subcutaneous route for 7 days in every 28 days. Cytarabine and azacitidine was administered 7 days at 20 mg/m^2 daily for 10 days sucutaneously and 75 mg/m^2 daily for 7 days, respectively. Both of the monotherapy and combination therapy was applied every 28 days for at least four cycles. Non-responders were administered decitabine at 20 mg/m^2 daily for 5 days or cytarabine at 100 mg/m^2 daily for 5 days, and idarubicine at 12 mg/m^2 daily was used as a second line of therapy. The patients who responded to therapy continued their prior chemotherapy protocol, which provided remission until the time of relapse. All patients received posaconazole at 200 mg tid as an antifungal prophylaxis and PO valacyclovir at 500 mg bid as an antiviral prophylaxis during the entire therapy process.

Assessment of the Response to Therapy

A whole blood count was performed before treatment, and a bone marrow biopsy, a bone marrow aspiration, and a flow cytometric analysis were conducted after the completion of 4 cycles of chemotherapy for each patient. The response to therapy was evaluated as a morphologic complete response (CR), a compete response with an incomplete blood count recovery (CRi), a partial response (PR), and irresponsiveness. The patients in both groups were compared regarding the overall survival, the response rates, the blast count in the bone marrow before and after treatment, the frequency of treatment-related complications, and the need for a blood transfusion during therapy. Progression free survival was defined as the beginning time of the diagnosis and at the time of relapsing and the beginning of the second line chemotherapy regimen. Overall survival was defined as beginning at the time of diagnosis and ending at the time of death. The surviving patients were censored.

Statistical Method

The SPSS 21.0 statistical package program was used for the statistical analysis. The descriptive statistics were presented as the number and the percent for categorical variables and presented as the mean, the standard deviation, the median, the minimum, and the maximum for numerical variables. For a comparison of the multiple independent groups, a qui-square test was used for paired and multiple comparisons when the qui-square condition was provided for the categorical variables, the Monte Carlo simulation was used for multiple comparisons, and the Fisher's exact test was used for paired groups. The survival analysis was conducted using the Kaplan–Meier method, and log-rank statistics were used for comparisons. The cox regression analysis was used with the Stepwise method for the multivariance analysis of the risk factors that were found to be significant in

the univarite Kaplan–Meier analysis (Fig. 1). The results were evaluated in a 95 % confidence interval, and a p level of <0.05 was accepted as statistically significant.

Results

Patient Characteristics

The mean age of the 27 patients was determined to be 71.30 ± 6.79 (mean ± SD). Of the patients, 12 (44.4 %) were female, and 15 (55.6 %) were male. Nineteen (70.37 %) were being followed up with a diagnosis of AML, and 8 (29.63 %) were being followed up with a diagnosis of MDA-RAEB2. The patients were evaluated separately as the azacitidine monotherapy group (51.9 %; n = 14) and the azacitidine + LD-ara-c combination therapy group (48,1 %; n = 13) and compared statistically. The patient characteristics of both groups are summarized in Table 1. While the metaphasis could not be provided in 5 (18.5 %) out of 13 (48 %) patients in which a cytogenetic evaluation was performed, a complex karyotype was detected in 4 (14.8 %), and a normal karyotype was found in 4 (14.8 %). Because of molecular studies couldn't studied in our center, we couldn't known the patients molecular status. A significant difference was not detected between the groups with regard to gender, survival, number of cycles, antibiotic-requiring infection, skin reaction. Number of patients who have more than 2 comorbidities were 5 in monotherapy group and 3 in combination group and this comorbidity numbers were not statistically different. The pre treatment blast percent of the patients in the azacitidine monotherapy group (31.430 ± 22.432) was lower than that of the azacitidine + LD-ara-c combination therapy group (51.310 ± 22.054) (p = 0.014). The platelet requirement was low in the azacitidine monotherapy group which was statistically significant in first 2 cycles and the second 2 cycles (2.360 ± 4.378 Units, 7.920 ± 6.788 Units, p = 0.004, 2.500 ± 8.528 Units, 7.080 ± 9.561 Units, p = 0.017 respectively).

The erythrocyte requriment during first 2 cycles and the second 2 cycles was higher in the patients who did not respond to first line therapy compared with that of the patients who responded to therapy (8.880 ± 4.443 Units, 5.580 ± 4.033 Units, p = 0.033,9.200 ± 6.026 Units, 0.920 ± 1.881 Units, p = 0.000, respectively).

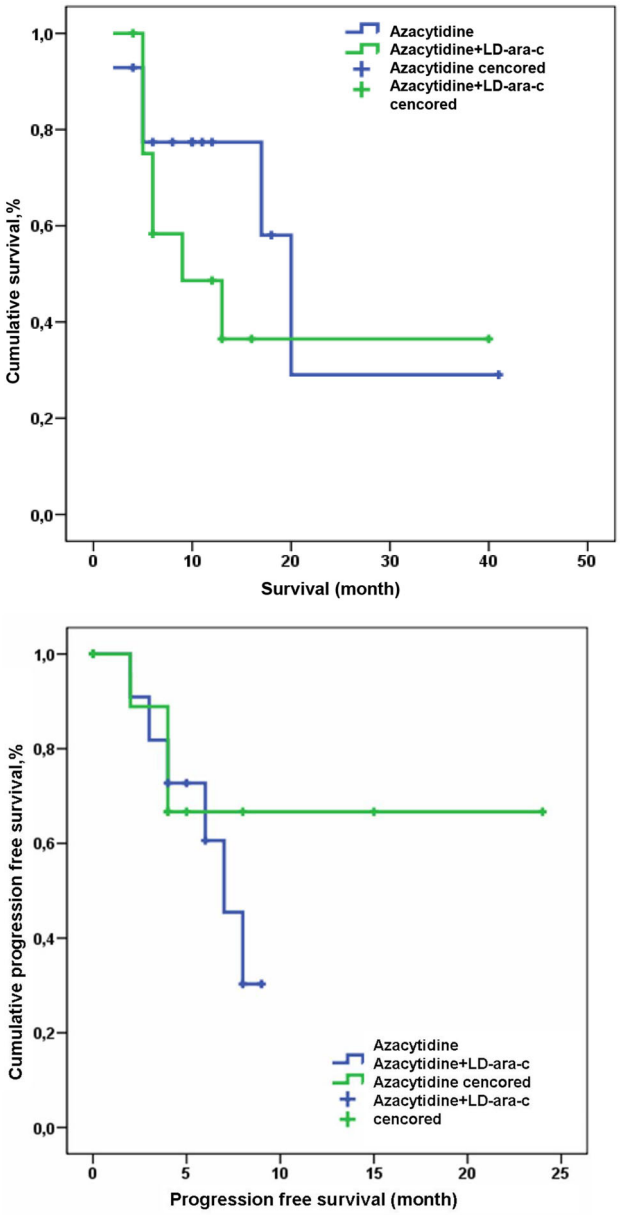

Fig. 1 Kaplan–Meier overall survival and progression free survival curves of the patients who used the monotherapy azacytidine and those who used the azacytidine + cytarabine combination therapy as the initial therapy

Response to the Treatment

The responses to first line therapy are summarized in Table 2. The overall response rate was found to be 42.9 and 57.1 % for the azacitidine monotherapy group and the combination therapy group, respectively, and a statistically significant difference was not detected between these two rates (p = 0.161). Five and four patients in the monotherapy and the combination therapy groups were received second line chemotherapy regiment. In monotherapy group, four patients were received decitabine an done patient was received 2 + 5 CT protocole because of acute leucemic transformation from MDS-RAEB2 with high blast count. In combination group, three patients were received 2 + 5 Ct protocole and one patient was received decitabine. One patient underwent a haploidentical bone marrow transplantation following the azacitidine treatment.

Table 1 Characteristics of patients who used azacytidine + LD-ara-c combination and who used azacytidine monotherapy as the initial therapy

Parameters	Azacytidine + LD-ara-c combination therapy ($N = 14$)	Azacytidine therapy ($N = 13$)	P
Age (year)	69.00 ± 6.014	73.430 ± 6.98	0.072
WBC before CT (μl)	10213,850 ± 18429,886	15027,640 ± 23125,509	0.264
WBC after CT (μl)	8892,380 ± 10433,890	4118,710 ± 5099,554	0.159
Hemoglobin before CT (g/dl)	8.206 ± 1.677	8.069 ± 1332	0.942
Hemoglobin after CT (g/dl)	10.185 ± 2.742	9.949 ± 2.345	0.846
Platelet before CT (μl)	54353,850 ± 40665,416	68558,570 ± 57031,948	0.771
Platelet after CT (μl)	92092,310 ± 85631,366	140282,140 ± 137635,654	0.593
ANC before CT (μl)	5578,690 ± 16146,351	3321,430 ± 7492,055	0.771
ANC after CT (μl)	1365,380 ± 1530,978	1630,500 ± 1444,255	0.422
Bone marrow blast before CT (%)	51.310 ± 22.054	31.430 ± 22.43	0.014*
Bone marrow blast after CT (%)	21.150 ± 24.72	7.570 ± 8.591	0.124
Number of CT (%)	4.640 ± 1.336	4.230 ± 0.832	0.326
E.S. transfusion (unit) during 1 + 2 cycles	8,620 ± 4,718	6.210 ± 4,098	0.213
E.S. transfusion (unit) during 3 + 4 cycles	6,690 ± 5,202	4.430 ± 7,046	0.154
P.S. transfusion (unit) during 1 + 2 cycles	7,920 ± 6,788	2,360 ± 4,378	0.004*
P.S. transfusion (unit) during 3 + 4 cycles	7,920 ± 9,561	2,500 ± 8,528	0.017*
Remission duration (month)	7,080 ± 6,959	6,690 ± 5,202	0.622
Overall survival time (month)	10,770 ± 9.619	12,790 ± 9,529	0.407

WBC white blood cells, *ANC* absolute neutrophil count *CT* chemotherapy, *E.S* erythrocyte suspension, *P.S* apheresis platelet suspension

Table 2 Responses of the patients who used azacytidine + LD-ara-c combination therapy and who used monotherapy azacytidine as the initial therapy

CR complete remission, *CRi* complete remission with incomplete blood count recovery

	Azacytidine n (%)	Azacytidine + LD-ara-c combination therapy n (%)	p
Response to therapy			0.161
Morphologic CR	5 (35,7)	4 (30,8)	
Cri	3 (4, 21)	–	
Partial remission	–	3 (23,1)	
Treatment failure	–	2 (15,4)	
Stable disease	6 (42,9)	4 (30,8)	

Treatment-Related Toxicity

The distribution of the toxicities based on the azacitidine monotherapy and the azacitidine-LD-ara-c combination treatment is summarized in Table 3. A statistically significant difference was not detected between the groups regarding the presence of antibiotic-requiring infection, skin reaction, need for additional antiemetic drugs, fever, duration of hospital stay due to infection, and the degree of neutopenia, leukopenia, thrombocytopenia, and anemia. Impaired liver function tests were not observed in the patients.

The median duration of the follow-up was determined to be 9.5 ± 9.628 (4–41 months) for all patients. The overall survival rate of the patients who received azacitidine monotherapy was 77.4 % for 1 year and 29.0 % for 2, 3, and 4 years, and it was 48.6 % for 1 year and 36.5 % for 2, 3, and 4 years in patients who received combination therapy. A statistically significant difference was not detected in the overall survival rates of the two groups ($p = 0.321$). The mean and the median of the overall survival rates and the follow-up are summarized in Table 4.

The progression-free survival rate was 30.3 % for 1 year in the monotherapy group, and 66.7 % for 1, 2, and 3 years in the combination group. A statistically significant difference was not detected in the survival rates of the groups ($p = 0.481$). The mean and the median of the overall survival rates and the follow-up duration of the treatment groups are summarized in Table 5.

The factors influencing the overall survival rate were evaluated. Overall survival was significantly longer in the patients who responded to first line therapy compared with the non-responders, who did not develop an infection

Table 3 Toxicities of the patients who used azacytidine + cytarabine combination and who used azacytidine monotherapy

	Azacytidine monotherapy (n, %)	Azacytidine + LD-ara-c combination therapy (n, %)	p
Antibiotic-requiring infection			
No	7 (50,0)	4 (30,8)	0.559
Yes	7 (50,0)	9 (69,2)	
Skin reaction			
No	5 (35,7)	2 (15,4)	0.487
Yes	9 (64,3)	11 (84,6)	
Nausea			
Grade 1	10 (71,4)	5 (38,5)	0.075
Grade 2	4 (28,6)	7 (53,8)	
Grade 3	0 (0,0)	1 (7,7)	
Infection			
No	6 (42,9)	2 (15,4)	0.393
Grade 1	–	2 (15,4)	
Grade 2	4 (28,6)	3 (23,1)	
Grade 3	2 (14,3)	6 (46,2)	
Grade 4	2 (14,3)	0 (0,0)	
Diarrhea			
No	11 (78,6)	10 (76,9)	0.841
Grade 1	3 (21,4)	2 (15,4)	
Grade 2	0 (0,0)	1 (7,7)	
Need for additional antiemetic drugs			
No	5 (35,7)	5 (38,5)	0.885
Yes	9 (64,3)	8 (61,5)	
Treatment-related fever			
No	8 (57,1)	5 (38,5)	0.341
Grade 1	6 (42,9)	8 (61,5)	
Leukopenia			
Grade 1	4 (28,6)	2 (15,4)	0.309
Grade 2	5 (35,7)	4 (30,8)	
Grade 3	5 (35,7)	7 (53,8)	
Thrombocytopenia			
Grade 1	6 (42,9)	3 (23,1)	0.073
Grade 2	4 (28,6)	1 (7,7)	
Grade 3	4 (28,6)	9 (69,2)	
Anemia			
Grade 1	3 (21,4)	2 (15,4)	0.130
Grade 2	5 (35,7)	1 (7,7)	
Grade 3	6 (42,9)	10 (76,9)	
Duration of hospital stay (Median ± SD, day)	10.140 ± 10.41	7.850 ± 11.77	0.341

compared with the patients whose grade of infection was greater than two and whose bone marrow blast ratio returned to normal ($p = 0.007$, $p = 0.008$, $p = 0.000$, respectively). Overall survival was significantly shorter in the patients who had fever compared with the patients without fever and who relapsed after 4 cycles of chemotherapy compared with the patients who did not relapse ($p = 0.037$, $p = 0.001$). The influence of other factors on the overall survival rate was not statistically significant. The data that were found to be significant in a univarite Kaplan–Meier analysis were analyzed using the cox regression analysis. The bone marrow blast count after chemotherapy was observed to be significant. The patients with a blast count of more than >20 % shortened overall survival 37.051 fold compare with the patients whose blast count was <5 %.

Table 4 Overall survival follow up durations of the patients in azacytidine monotherapy and azacytidine + LD-ara-c combination therapy group

	Mean				Median			
	Estimation	SE	95 % CI		Estimation	SE	95 % CI	
Azacytidine	21.59	5.23	11.038	32.774	20.000	3.8	15.425	24.575
Azacytidine + LD-ara-c	19.288	4.919	9.647	28.929	9.000	4.740	0.000	18.291
Overall survival of whole group of the patients	19.985	3084.003	12.140	27.830	17.000	4.851	7.492	26.508

p = 0.264

Table 5 Progression-free survival follow up durations of treatment groups

	Mean			
	Estimation	SE	95 % CI	
Azacytidine	6.545	0.755	5.065	8.025
Azacytidine + LD-ara-c	17.111	3.253	10.736	23.486
Progression-free survival of whole group of the patients	13.417	2.508	8.501	18.332

p = 0.481

Discussion

In this study, we aimed to compare azacitidine monotherapy and azacitidine + LD-ara-c combination therapies in newly diagnosed AML and RAEB2 patents who had a high blast count (>30 %) and who were not previously treated. To the best of our knowledge, this is the first study of this kind in the literature. The median duration of follow-up was determined to be 9.5 ± 9.628 (4–41 months). Median overall survival was not a statistically significant difference in both CT group (p = 0.407). The estimated median overall survival was higher in the monotherapy group but there was no statistically difference in both group. This situation can attributed to the small number of patients but on the other hand the patients who were in the combination group have more number of comorbidities and more blast counts. Besides the estimated progression free survival was longer in the combination therapy group despite the higher blast counts in the bone marrow. It may be related to the higher efficacy this treatment schedule but the larger studies must be planned. In a retrospective study conducted by Radujkovic et al., azacitidine and LD-ara-c treatments were compared, and response rates were determined to be 14 and 7 %, respectively [1]. In a multi-center study conducted with 155 AML patients, while the overall response rate was determined to be 52.3 %, the median overall survival as 9.8 months with azacitidine therapy, similarly to ours, these rates were reported as 33 % and 9.4 months in another multi-center study including 149 patients [12, 13]. Pleyer et al. reported in a large prospective trial that azacitidine can be safely and effectively used in elderly patients. In this study, 302 patients were evaluated. The overall response rate was 48 %, and the median overall survival was 9.6 months [14].

Radujkovic et al. reported in their study, while a statistically significant difference was not detected between the response to therapy rates and the toxicities, the one-year survival rate expectation was determined to be 15 and 13 % in comparing azacitidine and LD-ara-c treatments [11], respectively. Varying doses of cytarabine and azacitidine combination were used in combinations in a study conducted with an azacitidine and an LD-ara-c combination [5]. The overall response rate was reported to be 50 %, and the CR rate was reported to be 33.3 % following 2 cycles of therapy [5]. In another type of combination study was published in 2012. In this study, azacitidine and LD-ara-c combination therapy was administered in the same doses as in our study. The overall response rate was reported to be 50 %, and the median overall survival rate was reported to be 487 days [8]. Although the overall response rates are similar in this study and in our study, this situation may associate with a longer median survival with all patients being diagnosed as MDS [8]. In addition, various studies are available that used lenalidomide [15], panobinostate [16], bortezomib [17], and erlitinib [18] in combination with azacitidine in order to achieve better response rates in the elderly.

In our study, the factors influencing overall survival were found to be the response to first line therapy, the presence of grade 2 and above infection during treatment, and relapsing after treatment or being refractory to treatment in the multivariance analysis. According to previous studies, the cytogenetic factors determining the prognosis in AML patients were determined to be complete karyotype, MK positivity, 5.chromosome anomalies (−5, 5q-), 7.chromosome anomalies (−7, 7q-), 11q23abnl anomaly, inversion [3] and molecular factors such as an elevated expression of EVI1 (ectopic virus integration-1), an

absence of NPM1 mutation along with normal cytogenetics, and the presence of FLT3-ITD mutation [19]. We detected a complex karyotype in four (14.8 %) of our patients, but a statistical analysis was not conducted due to a small number of patients. Chen et al. reported that the pretreatment factors that influence prognosis were found to be a good performance score (Eastern Cooperative Oncology Group PS 0-1), LDH level (higher than 2 fold of normal), hyperleukocytosis (WBC $>$ 100 000/μl), significant thrombocytopenia ($<$ 20 000/μl) in 205 AML patients [20]. In a study conducted on 149 AML patients having a poor cytogenetic structure, WBC $>$ 15 000/μl, the ECOG performance score $\geq$2, and the response to therapy were determined to be the factors influencing overall survival [13].

When the side effects were analyzed in both treatment groups, a statistically significant difference was not detected between treatment-related neutropenia, thrombocytopenia, anemia, the amount of blood transfusion, grade of infection, diarrhea, skin reaction, hospitalization-requiring infection, and duration of hospital stay, and this is consistent with the literature [11, 21, 22].

The severity of infection (>grade 2 according to CTC), which was one of the most common problems in our patient group, was determined to be a factor influencing survival.

Conclusions

In conclusion, treatment responses, toxicities, and the factors influencing the survival of the azacitidine and the azacitidine + LD-ara-c combination groups were found to be consistent with the literature despite the small number of patients in our study. The response rates to therapy, overall survival, progression free survival and toxicities were not significantly different, although the pre-treatment bone marrow blast count was greater in the combination therapy group compared with the monotherapy group. We propose that this combination therapy may be a well-tolerated treatment option for the elderly, vulnerable AML patients whose blast count is high. Performing prospective studies with larger number of patients may be beneficial for determination of the results of the combination therapy in elderly patients.

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