Extramedullary acute myeloid leukemia presenting in young adults demonstrates sensitivity to high-dose anthracycline: a subset analysis from ECOG-ACRIN 1900

Extramedullary (EM) disease is an unusual manifestation of *de novo* acute myeloid leukemia (AML) occurring in less than 10% of patients at diagnosis. EM AML may manifest as cellular infiltrates of soft tissues (myeloid sarcomas), lymph nodes, skin (leukemia cutis) and leptomeninges, and is generally associated with a poor prognosis.[1-3] Therapeutic approaches are varied, but most include combination chemotherapy, radiation therapy and intrathecal therapy.[4-6] To obtain a better understanding of EM AML we evaluated a subset of younger (<61 years) patients, presenting with clinical EM disease at the time of diagnosis, who were treated as part of the Eastern Cooperative Oncology Group – American College of Radiology Imaging Network (ECOG-ACRIN) Cancer Research Group E1900 trial. We describe the clinical, chromosomal, molecular and immunophenotypic characteristics, and the outcomes following standard-dose versus high-dose daunorubicin in induction.

All patients were enrolled in the National Cancer Institute-approved trial (NCT00049517) conducted by the ECOG-ACRIN Leukemia Committee. From December 2002 through November 2008, a total of 657

Table 1. Baseline characteristics of patients with or without extramedullary disease.

| | Extramedullary Disease | | | | P |
| | No | | Yes | | |
	N	%	N	%	
Gender					0.78
Male	304	50.8	31	53.4	
Female	295	49.2	27	46.6	
Cytogenetics					0.47
Missing	2	0.3	0	0	
Favorable	84	14.0	5	8.6	
Indeterminate	156	26.0	20	34.5	
Intermediate	246	41.1	22	37.9	
Unfavorable	111	18.5	11	19.0	
FLT3-ITD					0.07
Unknown/missing	51	8.5	3	5.2	
Negative	420	70.1	36	62.1	
Positive	128	21.4	19	32.8	
MLL-PTD					0.02
Unknown/missing	40	6.7	0	0	
Negative	535	89.3	51	87.9	
Positive	24	4.0	7	12.1	
DNMT3A					0.05
Unknown/missing	155	25.9	12	20.7	
Negative	342	57.1	29	50.0	
Positive	102	17.0	17	29.3	
NPM1					0.22
Unknown/missing	144	24.0	11	19.0	
Negative	341	56.9	31	53.4	
Positive	114	19.0	16	27.6	
Total	599	100.0	58	100.0	

| | No | | | Yes | | | P |
	Median	Min	Max	Median	Min	Max	
Age (years)	48.0	17.0	60.0	41.5	18.0	60.0	0.01
Peripheral WBC count (x10⁹/L)	10.5	0.6	366.0	20.6	0.8	191.8	<0.001
Hemoglobin (g/dL)	9.2	4.6	30.0	8.9	6.0	15.4	0.76
Peripheral blasts (%)	30.0	0.0	99.0	40.0	0.0	94.0	0.05
Peripheral platelet count (x10⁹/L)	54.0	1.4	995.0	46.0	0.7	345.0	0.63
Marrow blasts (%)	64.0	8.0	100.0	70.0	3.0	100.0	0.22
CD56 expression	0.0	0.0	99.0	0.0	0.0	99.0	0.73

ITD: internal tandem duplication; PTD: partial tandem duplication; WBC: white blood cell.

patients with *de novo* untreated AML ranging in age from 17 to 60 years were enrolled. We relied on physicians' reporting of EM disease from clinical and diagnostic imaging findings. Disease in EM sites was not required to be biopsied nor were diagnostic imaging studies required to confirm involvement. Patients with isolated EM disease without bone marrow involvement were not eligible for this trial. The median follow-up of patients still alive is 6.7 years. Patients' bone marrow and peripheral blood samples were collected and sent to ECOG-ACRIN's leukemia reference laboratory for confirmation of the diagnosis of AML and immunophenotypic analysis by multiparameter flow cytometry. Internal tandem duplication (ITD) alterations in the Fms-like tyrosine kinase 3 (*FLT3*) gene and MLL partial tandem duplications (*MLL*-PTD) and mutational analysis profiling were performed on diagnostic samples from bone marrow and peripheral blood as previously described.[7-9] Cytogenetic data were reviewed by ECOG-ACRIN's Cytogenetics Committee. Data were collected and certified by the ECOG-ACRIN Data Coordinating Center and analyzed by the authors. The study was approved by the institutional review board at the National Cancer Institute and at each of the study centers. All patients provided written informed consent to participation in the study in accordance with the Declaration of Helsinki.

Eligible patients were randomly assigned to receive intravenous cytarabine 100 mg/m^2/day infused continuously for 7 days plus intravenous daunorubicin daily at a dose of either 45 mg/m^2 (standard dose) or 90 mg/m^2 (high dose) for 3 days with risk-adapted consolidation.[10]

For the statistical analysis, patients' baseline characteristics were compared using a Fisher exact test if the variables were categorical and Wilcoxon rank sum tests if they were continuous. Overall survival was defined as the time from randomization to death from any cause. For patients who achieved a complete response after induction, disease-free survival was defined as the time from documented complete response to relapse or death

from any cause. Kaplan-Meier estimates were used to estimate the event-time distributions for overall survival and disease-free survival.

Logistic regression models were used to examine the association between EM disease and complete response. The univariate model included indicators of whether patients had EM disease involvement and induction treatment arms. The multivariable model was further controlled for age, gender, cytogenetic risk, ECOG Performance Status, white blood cell count, platelet count, percentage of marrow blasts, hemoglobin, peripheral blast percentage, and secondary AML. Univariate and multivariable Cox models stratified on induction treatment arms were performed on overall survival and disease-free survival. The stratified univariate models included indicators of whether patients had EM disease involvement. The stratified multivariable models were further adjusted for age, gender, cytogenetic risk, ECOG Performance Status, white blood cell count, platelet count, marrow blast percentage, hemoglobin, peripheral blast percentage, secondary AML, *FLT*-ITD, and *MLL*-PTD mutations. All *P* values were based on two-sided tests.

Fifty-eight patients had EM disease; four patients had more than one site involved. Six patients had central nervous system involvement; 32 had gingival hypertrophy; two had a mediastinal mass and seven had skin involvement. Fifteen had involvement of other EM sites, including lung, lymphadenopathy and splenomegaly. Table 1 presents the baseline characteristics of patients with and without EM disease. Cytogenetic risk in the subgroup with EM did not differ statistically from that of patients without EM disease. Patients with EM AML were younger (median 41.5 *versus* 48 years; *P*=0.01) and had higher peripheral white blood counts (*P*<0.001) and peripheral blasts (*P*=0.05). Patients with EM AML had a higher incidence of expression of *MLL*-PTD (12.1% *versus* 4.0%; *P*=0.02). In the EM cohort, among the patients with mutation data available, 34.6% expressed

Log Rank Test p=0.25

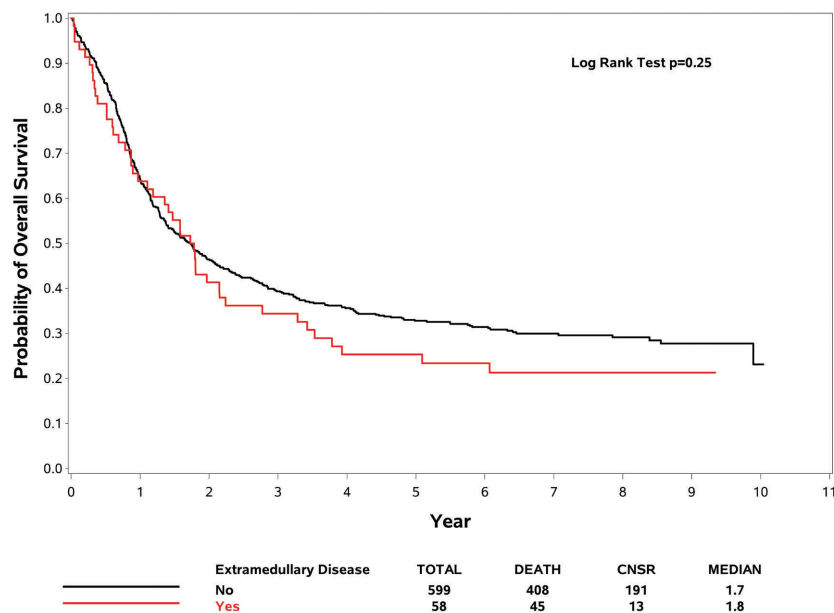| Extramedullary Disease	TOTAL	DEATH	CNSR	MEDIAN
No	599	408	191	1.7
Yes	58	45	13	1.8

Figure 1. Kaplan-Meier estimates of overall survival for patients with and without extramedullary disease. Data from the subset analysis are shown for overall survival of all patients with and without extramedullary disease treated in the trial. Data are from the time of randomization at the start of induction. CNSR indicates censored.

FLT3-ITD and 34.0% had *NPM1* mutations. These data were not statistically different from those in patients without EM disease (*P*=0.07 and *P*=0.22, respectively). Patients with EM AML had a higher incidence of *DNMT3A* gene mutations (37.0% *versus* 23.0%; *P*=0.05). The complete response rate with induction therapy of all patients with EM disease was 63.8%. The complete response rates in patients treated with standard-dose and high-dose daunorubicin induction therapy were 61.3% and 66.7%, respectively. When the two induction arms were combined, EM disease involvement did not significantly affect the complete response rate in both univariate (*P*=0.93) and multivariable models when controlling for other variables (*P*=0.95) There was no interaction of complete response rate between EM-disease-positive or -negative patients and the induction treatment (*P*=0.58).

For those patients who achieved a complete response after induction, EM involvement was not significantly associated with a worse disease-free survival in the univariate [hazard ratio (HR): 1.08, 95% confidence interval (95% CI): 0.73-1.61; *P*=0.70] or multivariable models (HR: 1.04, 95% CI: 0.65-1.64; *P*=0.88). There was no interaction of disease-free survival between whether or not patients had EM disease involvement and the induction treatment arms (*P*=0.83).

The probability curves of overall survival comparing the presence and absence of EM disease for the AML patients treated by induction arm (standard dose versus high dose) are given in Figures 1 and 2, respectively. When the two induction arms were combined, EM disease involvement was not significantly associated with a worse overall survival in comparison to that of patients without EM in univariate (HR: 1.20, 95% CI: 0.88-1.63; *P*=0.25) or multivariable models when controlling for other variables (HR: 1.21, 95% CI: 0.85-1.72; *P*=0.28). For patients randomized to receive 90 mg/m^2/day daunorubicin, whether or not patients had EM AML had no effect on overall survival (univariate model: HR: 1.00,

95% CI: 0.61-1.61; *P*=0.98; multivariable model: HR: 0.90, 95% CI: 0.51-1.58; *P*=0.72). Patients with EM AML treated with high-dose daunorubicin had a better overall survival that that of patients treated with standard-dose daunorubicin (median 2.1 *versus* 1.4 years; univariate model: HR: 0.54, 95% CI: 0.29-0.98; *P*=0.04; multivariable model: HR: 0.40, 95% CI: 0.15-1.07; *P*=0.07) (Figure 2).

In this subset analysis of EM AML patients from the E1900 study, higher doses of daunorubicin resulted in an improved overall survival compared to that in patients treated with standard-dose daunorubicin. These findings are consistent with our previously published results demonstrating that higher doses of anthracycline in induction therapy, followed by risk-adapted consolidation including hematopoietic cell transplantation, improves overall survival.[10,11] Based on our results and the sensitivity of EM AML, we now propose high-dose anthracycline in induction for these patients as for all those with *de novo* AML.

We noted significant differences in the EM AML population: these patients were younger, had higher white blood cell counts and circulating blasts, a higher incidence of *MLL*-PTD, and mutations of *NPM1* or *DNMT3A*. We noted a high incidence of the *NPM1* mutation (34.0%), which may have contributed to the favorable results of this analysis. *DNMT3A* mutations, an unfavorable prognostic factor, appeared in 31.7% of the EM AML population. *MLL*-PTD expression has previously been associated with a poor complete response rate, disease-free survival and overall survival.[12,13] Despite the presence of two adverse factors (*DNMT3A* and *MLL*-PTD) our patients' outcomes were better than expected, probably because of the positive effect of high-dose anthracycline noted in patients with these specific mutations.[9]

This is one of the largest reports of patients presenting with EM involvement concurrent with the diagnosis of *de*

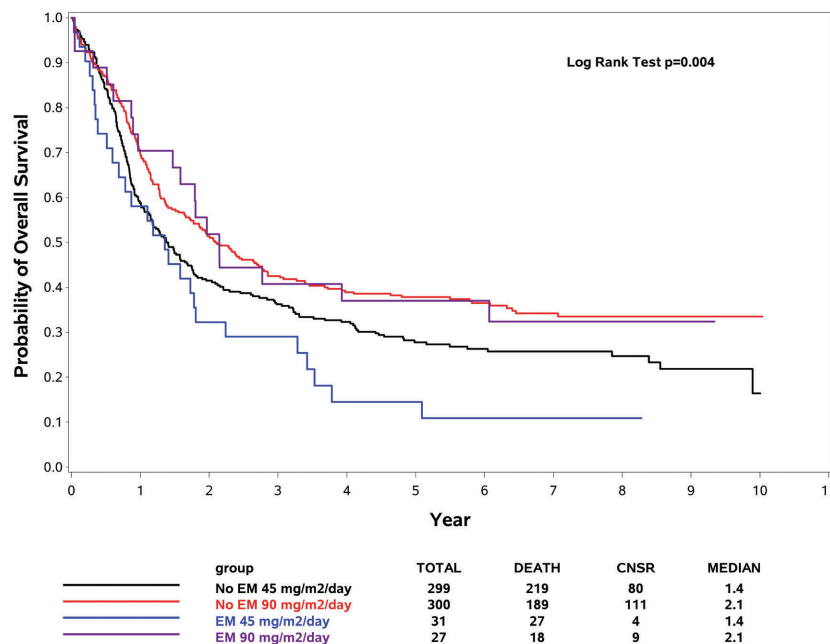| group	TOTAL	DEATH	CNSR	MEDIAN
No EM 45 mg/m2/day	299	219	80	1.4
No EM 90 mg/m2/day	300	189	111	2.1
EM 45 mg/m2/day	31	27	4	1.4
EM 90 mg/m2/day	27	18	9	2.1

Figure 2. Kaplan-Meier estimates of overall survival of patients with extramedullary disease based on induction therapy. Data from the subset analysis are shown for overall survival for patients with and without extramedullary disease who received protocol-prescribed induction therapy with a standard (45 mg/m^2) or high (90 mg/m^2) dose of daunorubicin. Data are from the time of randomization at the start of induction therapy. CNSR indicates censored.

novo AML. A major limitation of this analysis is that EM disease involvement had been noted clinically and/or detected by imaging studies and, therefore, there may have been other patients with undetected EM involvement who were not included in this analysis. Another limitation was this was not a planned subset analysis. A recently published, more encompassing retrospective analysis of prior ECOG-ACRIN trials demonstrated that AML with EM disease behaves similarly to that without EM involvement.[14] Thus, all patients with EM manifestations should be included in future prospective AML trials, as their outcomes are similar to those of patients without EM involvement. Future studies should continue to collect data and tissues from patients with EM AML in order to refine the therapeutic approach based on the available prognostic markers.

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