ARTICLE

Vosaroxin in combination with decitabine in newly diagnosed older patients with acute myeloid leukemia or high-risk myelodysplastic syndrome

Naval Daver,[1] Hagop Kantarjian,[1] Guillermo Garcia-Manero[1], Elias Jabbour,[1] Gautam Borthakur,[1] Mark Brandt,[1] Sherry Pierce,[1] Kenneth Vaughan,[1] Jing Ning,[2] Graciela M. Nogueras González,[2] Keyur Patel,[3] Jeffery Jorgensen,[3] Naveen Pemmaraju,[1] Tapan Kadia,[1] Marina Konopleva,[1] Michael Andreeff,[1] Courtney DiNardo,[1] Jorge Cortes,[1] Renee Ward,[4] Adam Craig[4] and Farhad Ravandi[1]

[1]Department of Leukemia, [2]Department of Biostatistics, and [3]Department of Hematopathology, The University of Texas M.D. Anderson Cancer Center, Houston, TX, USA and [4]Sunesis Pharmaceuticals Inc., South San Francisco, CA, USA

Haematologica 2017
Volume 102(10):1709-1717

ABSTRACT

Vosaroxin is an anti-cancer quinolone-derived DNA topoisomerase II inhibitor. We investigated vosaroxin with decitabine in patients ≥60 years of age with newly diagnosed acute myeloid leukemia (n=58) or myelodysplastic syndrome (≥10% blasts) (n=7) in a phase II non-randomized trial. The initial 22 patients received vosaroxin 90 mg/m^2 on days 1 and 4 with decitabine 20 mg/m^2 on days 1-5 every 4-6 weeks for up to seven cycles. Due to a high incidence of mucositis the subsequent 43 patients were given vosaroxin 70 mg/m^2 on days 1 and 4. These 65 patients, with a median age of 69 years (range, 60-78), some of whom with secondary leukemia (22%), adverse karyotype (35%), or *TP53* mutation (20%), are evaluable. The overall response rate was 74% including complete remission in 31 (48%), complete remission with incomplete platelet recovery in 11 (17%), and complete remission with incomplete count recovery in six (9%). The median number of cycles to response was one (range, 1-4). Grade 3/4 mucositis was noted in 17% of all patients. The 70 mg/m^2 induction dose of vosaroxin was associated with similar rates of overall response (74% *versus* 73%) and complete remission (51% *versus* 41%, *P*=0.44), reduced incidence of mucositis (30% *versus* 59%, *P*=0.02), reduced 8-week mortality (9% *versus* 23%; *P*=0.14), and improved median overall survival (14.6 months *versus* 5.5 months, *P*=0.007). Minimal residual disease-negative status by multiparametric flow-cytometry at response (± 3 months) was achieved in 21 of 39 (54%) evaluable responders and was associated with better median overall survival (34.0 months *versus* 8.3 months, *P*=0.023). In conclusion, the combination of vosaroxin with decitabine is effective and well tolerated at a dose of 70 mg/m^2 and warrants randomized prospective evaluation. ClinicalTrials.gov: NCT01893320

Introduction

Over two-thirds of patients with newly diagnosed acute myeloid leukemia (AML) in the United States of America (USA) and Europe are aged 65 years or older.[1-3] These older patients do not fare as well with intensive induction therapy, having complete remission (CR) rates <50%, a median survival of 4-9 months, and increased induction mortality (15-30%).[4-6] Hypomethylating agents (decitabine and azacytidine) are commonly used in the treatment of less fit, older patients with AML in the USA and Europe.[7] The pivotal DACO-016 study demonstrated a superior CR/CR without platelet recovery (CRp) rate with decitabine *versus* investiga-

Correspondence:

ndaver@mdanderson.org or
fravandi@mdanderson.org

Received: March 17, 2017.
Accepted: July 12, 2017.
Pre-published: July 20, 2017.

doi:10.3324/haematol.2017.168732

Check the online version for the most updated information on this article, online supplements, and information on authorship & disclosures: www.haematologica.org/content/102/10/1709

tors' choice of treatment (including low-dose cytarabine or best supportive care) in 485 older patients with AML (median age 73 years) who were ineligible for cytotoxic chemotherapy (17.8% *versus* 7.8%, *P*=0.001)[7] and improved survival with decitabine, leading to the approval of decitabine for the treatment of AML in the elderly in Europe.[7,8]

Vosaroxin is a non-anthracycline anticancer quinolone-derivative that intercalates DNA and inhibits topoisomerase II, causing site-selective DNA breaks, G2 arrest, and apoptosis.[9] Vosaroxin is not a substrate of P-glycoprotein-mediated efflux and can induce apoptosis independently of *P53* function.[9-11] In a phase II dose regimen optimization study in patients with previously untreated, unfavorable prognosis AML ≥60 years of age, single-agent vosaroxin resulted in a CR/CRp rate of 32%, a 30-day mortality of 12%, and a median survival of 7.0 months.[12] The 72 mg/m^2 days 1 and 4 and 90 mg/m^2 day 1 and 4 schedules of single-agent vosaroxin were well tolerated with the highest CR/CRp rates. The pivotal phase III, randomized, controlled, double-blind, multinational clinical study of the efficacy and safety of vosaroxin and cytarabine *versus* placebo and cytarabine in patients with first relapsed or refractory AML (VALOR) (n=711) demonstrated that vosaroxin in combination with intermediate-dose cytarabine produced a significantly superior remission rate (30% *versus* 16%; *P*<0.0001) and improved overall survival (OS) with equivalent 60-day mortality as that following cytarabine alone.[13] The overall survival benefit with the combinations was most prominent in patients older than 60 years (7.1 months *versus* 5.0 months, *P*=0.003).

The non-confluent safety profile of vosaroxin and decitabine, their non-overlapping molecular mechanisms of action, and the encouraging data with vosaroxin alone, and vosaroxin in combination with cytarabine in the older AML population lent support to this phase II trial of vosaroxin with decitabine in untreated elderly patients (≥60 years) with AML or high-risk myelodysplastic syndrome (MDS) unsuitable for intensive induction. This study was designed to assess whether the addition of vosaroxin to decitabine can improve response rates and OS compared to established outcomes with decitabine alone while maintaining an acceptable safety profile. The decitabine dose and schedule were those used in AML registration studies in the USA and Europe[7] and in published phase II clinical studies with decitabine alone[14] or in combination with idarubicin, amsacrine or daunorubicin.[15,16] The vosaroxin dose and schedule were selected from the phase II study of frontline vosaroxin in older AML patients.[12]

Methods

Patients' eligibility

Eligible patients were subjects ≥60 years of age with untreated AML or untreated high-risk MDS (intermediate-2 or high according to the International Prognostic Scoring System and ≥10% blasts) who were unsuitable for standard induction in the opinion of the treating physician. Non-suitability for induction chemotherapy was based on the predictive prognostic model for outcome in older patients with AML published by Kantarjian *et al.*[4] Patients with an Eastern Cooperative Oncology Group performance status ≤3; serum creatinine ≤2.0 mg/dL; serum bilirubin ≤2.0 mg/dL; serum transaminase ≤2.5 times the upper limit of the normal range or ≤5 times upper limit of the normal range if the transaminase elevation was deemed related to leukemic infiltration, were enrolled on the study. This was a single-center, open-label, non-randomized study. All patients signed an informed consent form approved by the University of Texas - M. D. Anderson Cancer Center (UT/MDACC) Institutional Review Board. The study was conducted in accordance with the Declaration of Helsinki. (ClinicalTrials.gov identifier: NCT01893320)

Study design and objectives

This study recruited patients between 15 September, 2013 and 23 May, 2016. A total of 65 patients were enrolled. The latest follow-up date was 20 September, 2016. The primary trial endpoint was to establish the safety and efficacy [overall response rate (ORR) = CR, CRp or CR with incomplete recovery of peripheral counts (CRi) assessed as the best response achieved on study] of the combination. Secondary endpoints included analysis of the OS, event-free survival, toxicities, and the correlation of outcomes to baseline cytogenetic and molecular profiles.

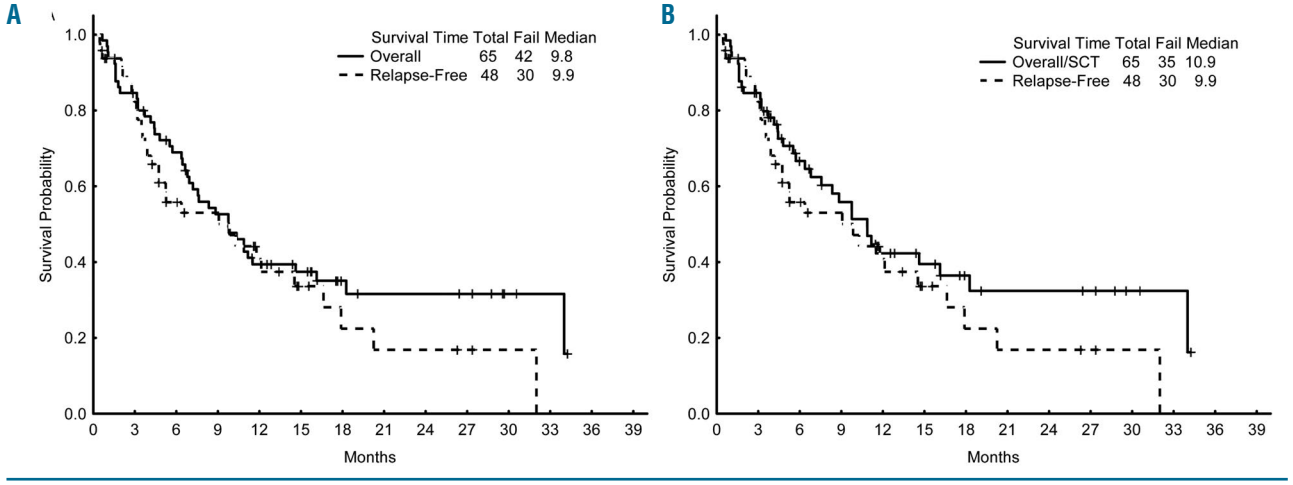

Figure 1. Survival and relapse-free survival in all patients on study. (A) Survival and relapse-free survival among all patients treated with vosaroxin in combination with decitabine on trial not censored and (B) censored for allogeneic stem cell transplant (SCT).

Treatment regimen

The induction regimen included 5 days of decitabine at a dose of 20 mg/m^2 given intravenously (IV) over 60 to 90 min. The vosaroxin was initially administered at a dose of 90 mg/m^2 to 22 patients (patients #1-22) on days 1 and 4 (Figure 1). Grade 3/4 mucositis was noted in five of these 22 (23%) patients, prompting a dose reduction of vosaroxin. The next 43 patients (patients #23-65) received vosaroxin 70 mg/m^2 on days 1 and 4. Patients underwent bone marrow aspiration on day 28 (±5) days. Patients whose day 28 bone marrow showed ≥5% blasts received re-induction with the same dose and schedule as the induction. Patients who did not achieve morphological remission (<5% blasts) at the end of course 1 had a repeat bone marrow examination at the end of course 2. Patients with a response or clinical benefit after one or two induction courses received post-induction therapy with up to five additional cycles of the combination with decitabine 20 mg/m^2 on days 1-5 and either vosaroxin 70 mg/m^2 on days 1 and 4 or a reduced dose of vosaroxin of 50 mg/m^2 or lower, based on response including minimal residual disease (MRD) status, toxicity, and count recovery. Post-induction cycles were repeated every 4-6 weeks, depending on count recovery and resolution of other toxicity. Bone marrow aspirations were repeated every three to four courses while on therapy. Patients who maintained a response (CR or CRp or CRi) at the end of post-induction therapy could receive maintenance with decitabine alone every 4-6 weeks for up to 24 additional cycles.

Baseline assessments

Pretreatment evaluations included complete history and physical examination, complete blood count with differential, a comprehensive biochemistry panel, pregnancy test and counseling, and bone marrow aspiration for histological, multiparametric flow-cytometric, cytogenetic analyses, and next-generation sequencing. Multiparametric flow-cytometry and cytogenetics were performed at our institution.[17,18] A next-generation sequencing-based analysis for the detection of somatic mutations in the coding sequences of 28 genes was performed on DNA extracted from the bone marrow sample. The methodology of our mutation analysis panel and coverage by genes has been previously published[19] (*Online Supplementary Table S1*).

Response criteria and definitions

Responses were according to established criteria for AML and included the best response achieved on study.[20,21]

Toxicity assessment

In the lead-in portion of the study, the safety and tolerable dose of the combination were assessed to identify the maximum tolerated doses. Six patients were to be treated in the lead-in portion. If clinically significant, drug-related grade 3-4 toxicity was observed during the first 28 days on therapy in one or none of six patients, this would define a safe schedule and the study would proceed to expansion. If study drug-related grade 3-4 toxicity was observed in two or more of six patients during the first 28 days, this dose would exceed the maximum tolerated dose, and a lower dose schedule would be investigated. The dosing algorithm is presented in Table 1. The maximum tolerated dose was considered as the highest dose level at which fewer than two of six patients developed dose-limiting toxicity in the first 28 days on therapy.

In the phase II portion of the study, patients were monitored continuously for toxicity.[22] We denoted the probability of toxicity by θ_E, where toxicity was defined as any clinically significant grade 3 or 4 non-hematologic toxic effect or death, according to the Common Terminology Criteria for Adverse Events version 4.0, attributable to the study drug. We assumed non-informative toxicity prior of beta θ_E ~ beta (0.3, 1.7). The stopping rule was given by the following probability statement: P (θ_E >0.15 | data) >0.90. That is, we would stop the trial if, at any time during the study, we determined that there was a more than 90% chance that the toxicity rate would be greater than 15%.

Statistical methods

The expected response rate with single-agent decitabine in this population of patients is 18-40%.[7,14] Under a null hypothesis of 40% with decitabine alone, a sample size of 59 patients would have more than 80% power to detect a difference between a response rate of 60% for the combination of vosaroxin and decitabine and the null response rate using a one-sample exact binomial test with a two-sided alpha of 0.05.

In the expansion phase of the study, patients were monitored continuously for futility. The ORR was assumed to follow a non-informative prior of beta (1.2, 0.8). The stopping boundaries for ORR were that if, at any time during the study, we determined that there was a less than 2.5% chance that the ORR was greater than 60%, we would terminate the study.[22]

Differences among variables were evaluated by the chi-square test (or Fisher exact test for cell frequencies <5) for categorical variables and *t*-test or Wilcoxon-Mann-Whitney test for continuous variables. Survival distributions were estimated using Kaplan–Meier methods and compared using the log-rank test.[23] All P values were two-sided and P<0.05 was considered statistically significant. Statistical analyses were carried out using IBM SPSS Statistics 21 for Windows (SPSS Inc., Chicago, IL, USA).

Results

Patients' characteristics

The first six patients in the lead-in portion were treated at dose level 0 (Table 1), receiving decitabine 20 mg/m^2 on days 1-5 and vosaroxin 90 mg/m^2 on days 1 and 4. There were no documented dose-limiting toxicities during the first 28 days in the first six patients and the study opened broadly for expansion at this dose. In the expansion phase 16 additional patients received the combination with vosaroxin 90 mg/m^2 on days 1 and 4 and grade 3/4 mucositis was noted in five of these 16 patients (31%). The high incidence of mucositis prompted amendment of the protocol to reduce the vosaroxin dose to 70 mg/m^2 on days 1 and 4. The subsequent 43 patients (patients #23-65) received decitabine 20 mg/m^2 on days 1-5 and vosaroxin at a dose of 70 mg/m^2 on days 1 and 4.

The median age of the patients was 69 years (range, 60-78) and 40% of them were older than 70 years of age. Their pre-treatment clinical characteristics are summarized in Table 2. Fifty-eight patients (89%) had AML and seven had MDS. One-third (35%) of the patients had complex cytogenetics. A clinically validated next-generation sequencing-based analysis was performed in 63 of the

Table 1. Protocol dosing algorithm.

Dose level	Vosaroxin Days 1 and 4	Decitabine
0	90 mg/m^2	20 mg/m^2 (Days 1-5)
-1	70 mg/m^2	20 mg/m^2 (Days 1-5)
-2	50 mg/m^2	20 mg/m^2 (Days 1-5)
-3	50 mg/m^2	20 mg/m^2 (Days 1-4)

65 (97%) patients. *TP53* (20%), *IDH2* (18%), *TET2* (15%), and *K/N-RAS* (18%) were the most frequent mutations.

Response to therapy

All 65 patients are evaluable for response. The ORR among the 65 patients was 74%, including 31 CR (48%), 11 CRp (17%), and six CRi (9%). The median number of cycles to response was one (range, 1-4). Ten patients (15%) were primary refractory. Deaths were documented in one (2%) and nine (14%) patients at 4 and 8 weeks, respectively. MRD was assessed by multiplanar flow cytometry at the time of response (± 3 months) in 39 of the 48 responders (81%) including 25 of the 32 (78%) responders at the 70 mg/m^2 dose and 14 of the 16 (88%) responders at the 90 mg/m^2 dose. MRD was not detectable in 21 of the 39 (54%) patients evaluated.

We assessed response by patient's age at enrollment, baseline cytogenetics and molecular profile (Table 3). The response rates were not significantly different among patients 60-74 years of age and those ≥75 years of age. Patients with an adverse karyotype (including complex karyotype and abnormalities of chromosome 5 and/or 7) at baseline had a response rate of 65%, as compared to 79% in patients with diploid or other non-adverse karyotype. The response rate among the 13 patients with mutated *TP53* was 77%. Three patients had been given prior hypomethylating agent (HMA) therapy for MDS or MDS/myeloproliferative neoplasm and went on to receive decitabine with vosaroxin at the time of progression to AML. Two of the three achieved a response (1 CRp and 1 CRi) indicating that the response rate in patients previously treated with HMA was comparable to that in the entire study population.

We compared the outcomes of the 22 patients (patients 1-22) who received vosaroxin 90 mg/m^2 in their induction course to the 43 patients (patients #23-65) who received vosaroxin 70 mg/m^2 (Table 4). The 8-week mortality was lower with the lower dose of vosaroxin (23% *versus* 9%; *P*=0.14) and the response rates were similar (ORR = 73% *versus* 74%, CR = 41% *versus* 51%; *P*=0.435). Of note, a higher proportion of patients required more than one cycle of induction to achieve a response with the 70 mg/m^2 dose, with 19 (59%) achieving a response after one course, nine (28%) after two courses, and four (13%) after three courses. Among the 16 responders at the 90 mg/m^2 induction dose, 13 (81%) achieved a response after one course, one (6%) after two courses, and two (13%) after three courses. Individual patient's response status, survival, and disposition of the 48 responders are provided in a swim plot (*Online Supplementary Figure S1*).

A number of patients who were not considered to be transplant candidates at the time of induction had improvement in their physical condition after achieving remission and could be considered for an allogeneic stem cell transplant (ASCT). Twelve patients proceeded to ASCT, including two of 16 (13%) responders given the vosaroxin 90 mg/m^2 dose and ten of 32 (31%) responders on the vosaroxin 70 mg/m^2 dose. The median time from the start of therapy to ASCT was 3.9 months (range, 1.8 –

Table 2. Characteristics of the study population (n=65).

Characteristic	Category	N (%); Median [range]
Age (years)		69 [60-78]
	60-69	38 (58)
	≥70	27 (42)
Diagnosis	AML – *de novo*	44 (68)
	Secondary AML	14 (22)
	HR MDS	7 (11)
	Secondary MDS	0 (0)
Prior Rx for AHD	HMA	3 (5)
	Lenalidomide	1 (2)
BM blast %		36 [9-97]
WBC x10^9/L		3.6 [0.4-57.0]
Platelets x10^9/L		36 [7-333]
Cytogenetics	Diploid	24 (37)
	Miscellaneous	15 (23)
	-5/-7/complex	23 (35)
	Insufficient	3 (5)
Mutation status (n=65)	*TP53*	13 (20)
	IDH2	12 (18)
	IDH1	9 (14)
	TET2	10 (15)
	RAS (K/N)	12 (18)
	DNMT3A	8 (12)
	CEBPA	8 (12)
	ASXL1	8 (12)
	JAK2	3 (5)
	FLT3	4 (6)
	EZH2	2 (3)

N/n: number; %: percentage; AML: acute myeloid leukemia; HR: high risk; MDS: myeloid dysplastic syndrome; Rx: treatment; AHD: antecedent hematologic disorder; HMA: hypomethylating agent; BM: bone marrow; WBC: white blood cell count.

Table 3. Response by baseline characteristics of the patients (n=65).

Parameter	Category	N	Overall response (CR, CRp, CRi)	CR
Age (years)	60-74	52	75%	50%
	≥75	13	69%	38%
Cytogenetics	Diploid	24	79%	54%
	-5/-7/other adverse	23	65%	35%
	Miscellaneous	18	78%	56%
Mutation status	*IDH2*	12	92%	75%
	IDH1	9	33%	33%
	TP53	13	77%	46%
	RAS	12	58%	17%

N: number; CR: complete remission; CRp,: complete remission with incomplete platelet recovery; CRi,: complete remission with incomplete blood count recovery.

Table 4. Outcomes by induction dose of vosaroxin, N=65.

Induction dose (vosaroxin)	N	Median overall survival	8-week mortality	Overall response (CR, CRp, CRi)	Complete remission	Need >1 cycle to response
90 mg/m^2	22	5.5 months	23%	73%	41%	3 (19%)
70 mg/m^2	43	14.6 months	9%	74%	51%	13 (41%)

N: number; CR. complete remission; CRp: complete remission with incomplete platelet recovery; CRi: complete remission with incomplete recovery of blood counts.

7.6). All patients were in CR/CRp/CRi at the time of transplantation. Six of the ASCT donors were matched siblings, the other six were matched, unrelated donors.

Remission duration and survival

With a median follow up of 17.5 months (range, 3.6-34.2), 23 patients are alive and nine are in remission. The median OS for all 65 patients is 9.8 months (range, 0.7 – 34.2) with 1-year and 2-year OS rates of 39% and 32%, respectively (Figure 1A). The median OS censored for patients who underwent ASCT was 10.9 months (range, 0.7 – 34.2) with 1-year and 2-year censored OS rates of 42% and 32%, respectively (Figure 1B).

Of interest, patients treated with the 70 mg/m^2 dose of vosaroxin at induction had a significantly longer median OS than those treated with the 90 mg/m^2 dose (14.6 months *versus* 5.5 months, *P*=0.007) (Figure 2A). The 70 mg/m^2 induction dose was associated with a significantly improved 1-year survival (51% *versus* 18%) and 2-year survival (44% *versus* 16%). Furthermore, patients treated with the 70 mg/m^2 induction dose continued to fare better with censoring for ASCT (16.1 months *versus* 5.5 months, *P*=0.01) (Figure 2B). Patients 60-74 years of age had a significantly longer median OS with the vosaroxin 70 mg/m^2 induction dose than with the vosaroxin 90 mg/m^2 induction dose (16.1 months *versus* 7.6 months, *P*=0.025) (Figure 2C). Similarly, patients ≥75 years of age had a longer median OS with vosaroxin 70 mg/m^2 induction as compared to vosaroxin 90 mg/m^2 induction (5.7 months *versus* 1.6 months, *P*=0.06) (Figure 2D).

Patients with complex cytogenetics had a shorter median OS than those with diploid or miscellaneous cytogenetics (5.7 months *versus* 34.0 months *versus* 9.8 months, *P*=0.003) (Figure 3A). Patients treated with vosaroxin 70 mg/m^2 with complex cytogenetics had a median OS of 6.6 months and this remained inferior to that of patients with diploid or miscellaneous cytogenetics treated at the same dose (6.6 months *versus* median not reached, *P*=0.011) (Figure 3B). The patients with *TP53* mutations (n=13) had a median OS of 5.7 months as compared to a median OS of 11.2 months in patients without a *TP53* mutation (n=51) (*P*=0.01). Patients with *TP53* mutations who were treated with the 70 mg/m^2 induction dose of vosaroxin (n=7) had a median OS of 7.2 months compared to the 1.8 months of those treated with the 90 mg/m^2 dose (n=6) (*P*=0.03) (Figure 3C). Three patients had received prior HMA therapy for MDS and were enrolled on decitabine and vosaroxin at progression to AML. The median OS of these three patients was 4.4 months which is significantly shorter than the median OS of 9.8 months achieved in the entire study population, although the number for comparison is small.

The median numbers of decitabine + vosaroxin cycles received by the 48 responders (CR/CRp/CRi) and the 17 non-responders were three (range, 1 – 7) and one (range, 1 – 3), respectively. The median numbers of cycles received by patients who achieved CR, CRp, and CRi were four (range, 2 – 7), three (range, 1 – 6), and two (range, 1 – 3), respectively.

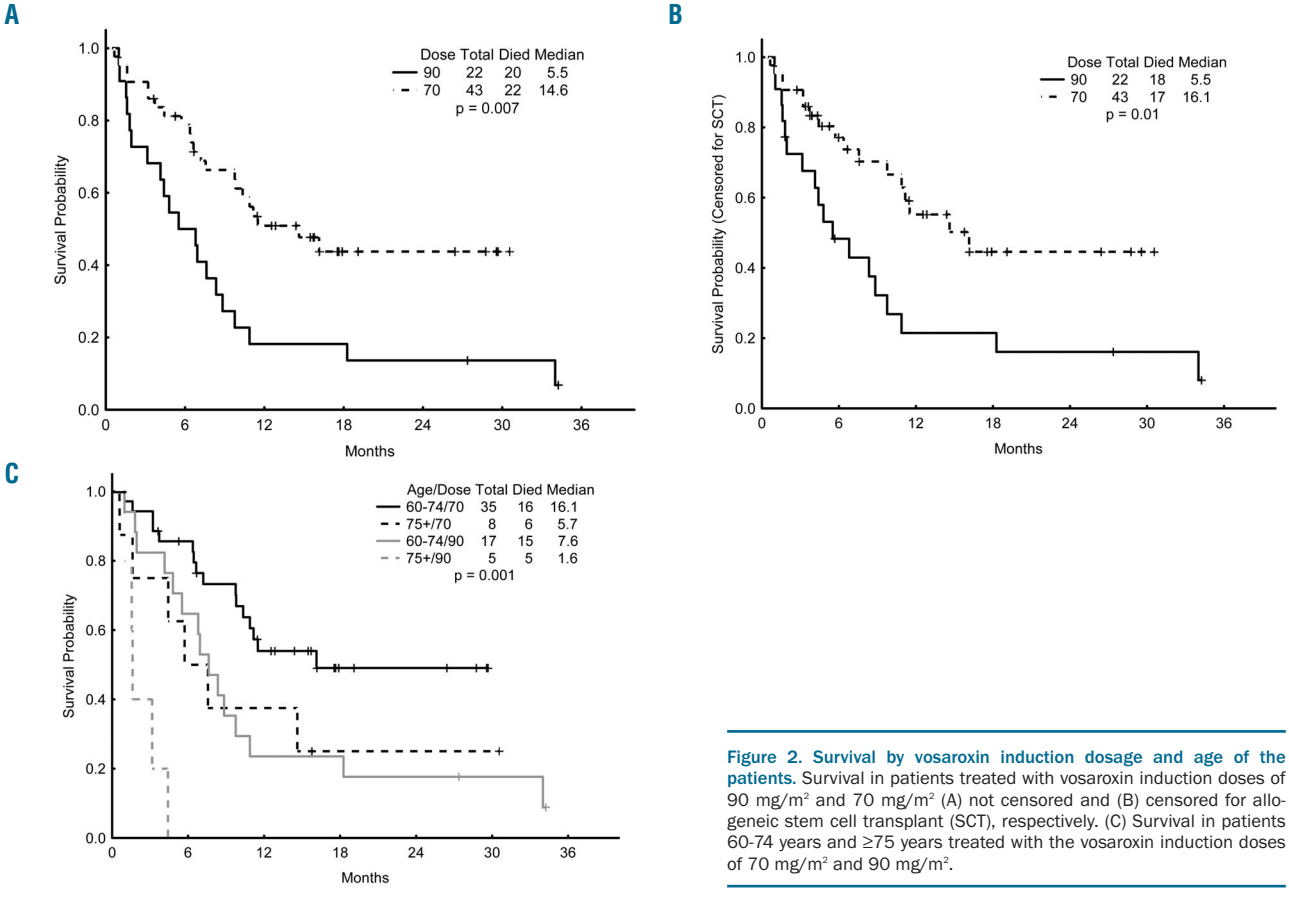

Figure 2. Survival by vosaroxin induction dosage and age of the patients. Survival in patients treated with vosaroxin induction doses of 90 mg/m^2 and 70 mg/m^2 (A) not censored and (B) censored for allogeneic stem cell transplant (SCT), respectively. (C) Survival in patients 60-74 years and ≥75 years treated with the vosaroxin induction doses of 70 mg/m^2 and 90 mg/m^2.

To evaluate the impact of the number of cycles of decitabine and vosaroxin among the 48 responders (CR/CRp/CRi) we analyzed OS according to whether the patients received more or less than the median number of cycles of the combination (median number of cycles of decitabine and vosaroxin in the 48 responders was 3). The median OS was significantly shorter in responders who received three or fewer cycles of the combination than in responders who received four or more cycles of the combination (6 months *versus* 34 months, $P<0.001$).

We performed the same analysis among the 31 patients who achieved a CR (median number of cycles of decitabine and vosaroxin in the 31 CR patients was 4). The median OS was significantly shorter in patients with CR who received four or fewer cycles of the combination than in responders who received five or more cycles of the combination (6 months *versus* 34 months, $P<0.001$).

Minimal residual disease at response

Responders who achieved MRD-negative status at the time of their response (± 3 months) had a significantly longer OS than those who remained MRD-positive at response (34.0 months *versus* 8.3 months, $P=0.023$). Thirteen of 25 (52%) evaluable responders treated at the 70 mg/m^2 dose achieved MRD-negative status at response and had a significantly improved survival as compared to those who remained MRD-positive (median not reached *versus* 7.6 months, $P=0.006$). Eight of 14 (57%) evaluable responders treated at the 90 mg/m^2 dose achieved MRD-negative status at response but this was not associated with improved survival (6.9 *versus* 8.3 months, $P=0.81$) (Figure 4A-C). We compared baseline clinical characteristics (age, vosaroxin induction dose, *de novo*/secondary AML, cytogenetic group, bone marrow blasts, platelet count, mutated/non-mutated *TP53*) among responders with MRD evaluable at response to see whether any influenced the achievement of MRD-negative status (*Online Supplementary Table S2*). None of the baseline features was predictive for achievement of MRD negativity.

The patients who achieved CR had a longer overall survival than the patients who achieved CRp or CRi (18.3 months *versus* 9.8 months *versus* 4.4 months, $P=0.023$) (*Online Supplementary Figures S2 and S3*).

Safety and tolerability

The most common drug-related toxicity was mucositis

Table 5. Toxicities in the study patients (n=65).

Toxicities	Grade 1/2 N (%)	Grade 3/4 N (%)	Total N (%)
Mucositis	26 (40)	11 (17)	37 (57)
Bilirubin	21 (32)	8 (12)	29 (45)
Nausea/vomiting	8 (12)	1 (2)	9 (14)
Diarrhea	2 (3)	0 (0)	2 (3)
Major infections (pneumonia, sepsis)	NA	46 (71)	46 (71)
Other site infections	NA	6 (9)	6 (9)
Fungal infections	NA	2 (3)	

N/n: number; %: percentage.

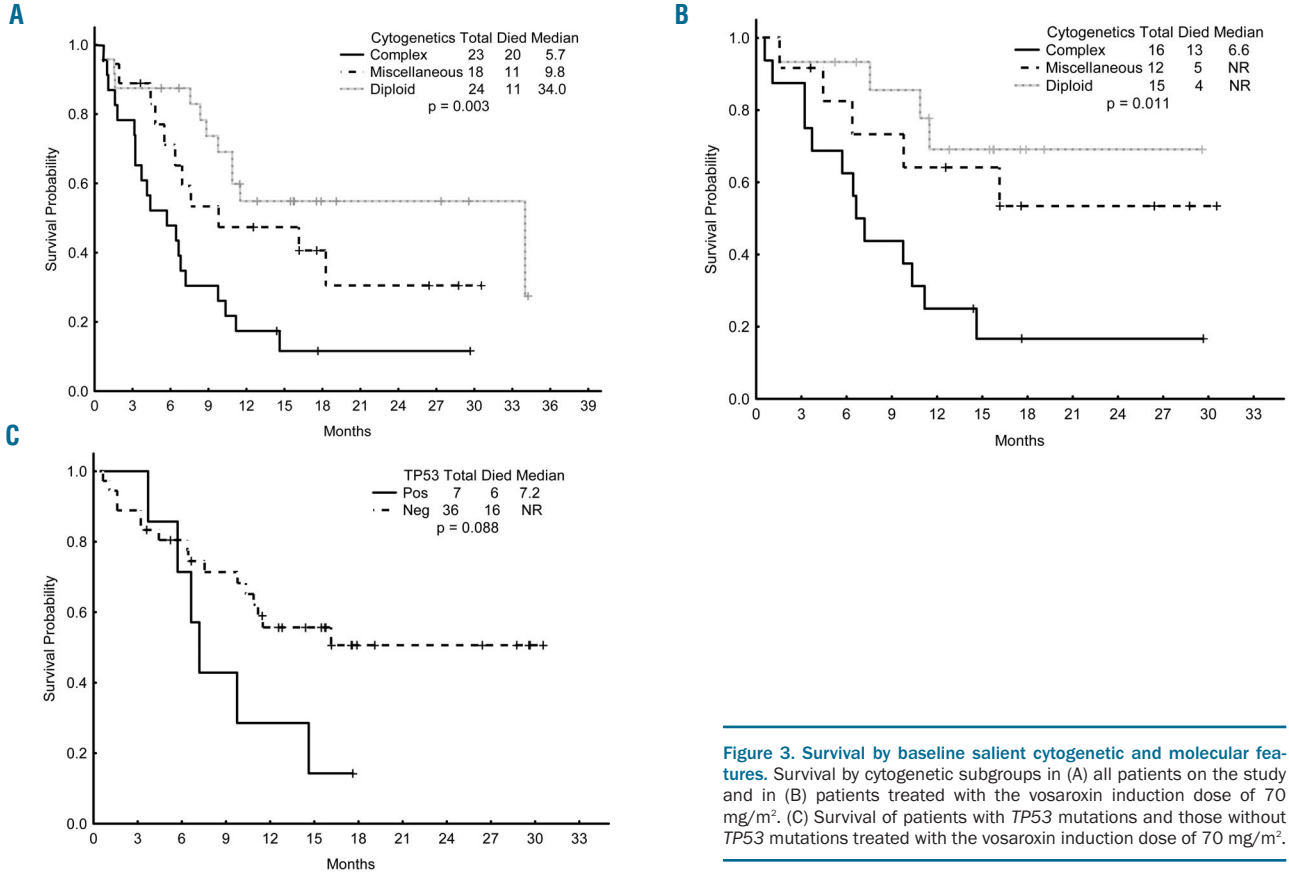

Figure 3. Survival by baseline salient cytogenetic and molecular features. Survival by cytogenetic subgroups in (A) all patients on the study and in (B) patients treated with the vosaroxin induction dose of 70 mg/m^2. (C) Survival of patients with *TP53* mutations and those without *TP53* mutations treated with the vosaroxin induction dose of 70 mg/m^2.

(Table 5). Grade 3/4 mucositis was seen in 11 (17%) patients and grade 1/2 mucositis in 26 (40%). With regards to the frequency and severity of the mucositis according to induction dose, grade 3/4 mucositis occurred in 16% *versus* 23% of the patients given 70 mg/m^2 or 90 mg/m^2, respectively (P=0.85), whereas grade 1/2 mucositis occurred in 30% *versus* 59%, respectively (P=0.02). Other drug-related toxicities included elevated levels of bilirubin in 45% of the patients: 11 (26%) of those given 70 mg/m^2 and 18 (82%) of those given 90 mg/m^2 (P<0.001). Most of these were grade 1/2 events and resolved. The rate and distribution of infectious complications were as expected for elderly AML patients receiving an induction regimen.

Seven of the 12 (58%) patients who underwent ASCT have died from bone marrow relapse (n=3), central nervous system relapse (n=1) and post-transplant infections (n=3; all 3 died in CR). Forty-two of the 65 (65%) enrolled patients have died by the time of making this report. The causes/timing of death were induction-related (within 8 weeks) in nine (21%) cases, relapsed/refractory AML in 22 (52%), post-transplant in seven (17%), infection while in CR/CRp/CRi in two (5%), and unknown (death after leaving MD Anderson) in another two (5%). Of the 13 patients with mutated *TP53* treated on the trial, one remains alive. The causes/timing of death in the remaining 12 were induction-related (within 8 weeks) in three (25%) cases, relapsed/refractory AML in six (50%), post-transplant in two (17%), and unknown in one.

Discussion

The initial 22 patients on our study received vosaroxin at the induction dose of 90 mg/m^2 on days 1 and 4. We observed a high incidence of mucositis and early mortality (8-week mortality rate, 23%) from neutropenic infections likely related to mucositis. The 70 mg/m^2 dose was then evaluated in the subsequent 43 patients with a reduction in the incidence of overall mucositis and early mortality (8-week mortality rate, 9%). This led to an improved OS in the cohort treated with the 70 mg/m^2 dose.

Older patients with AML more frequently have an antecedent hematologic disorder, unfavorable cytogenetics, and poorer performance status at presentation.[4,5] As a result, the outcomes of elderly patients with AML has not improved significantly over the last four decades.[3,24,25] Overall, standard induction regimens can achieve CR/CRp rates of 45-50%, median survival of 4-9 months with an 8-week mortality rate of up to 30%.[4-6,10] These dismal outcomes have resulted in a shift over the last decade to lower intensity strategies such as HMA or low-dose cytarabine in Europe and the USA. Phase II and III trials of decitabine in elderly patients with AML have shown CR/CRp rates of 18-25% with median survival of 7-8 months and 60-day mortality rates of 10-18%.[7,14] In the DACO-016 trial the response rate, median survival, and 60-day mortality in the low-dose cytarabine or supportive care comparator arm were inferior to those in the decitabine arm. In an effort to

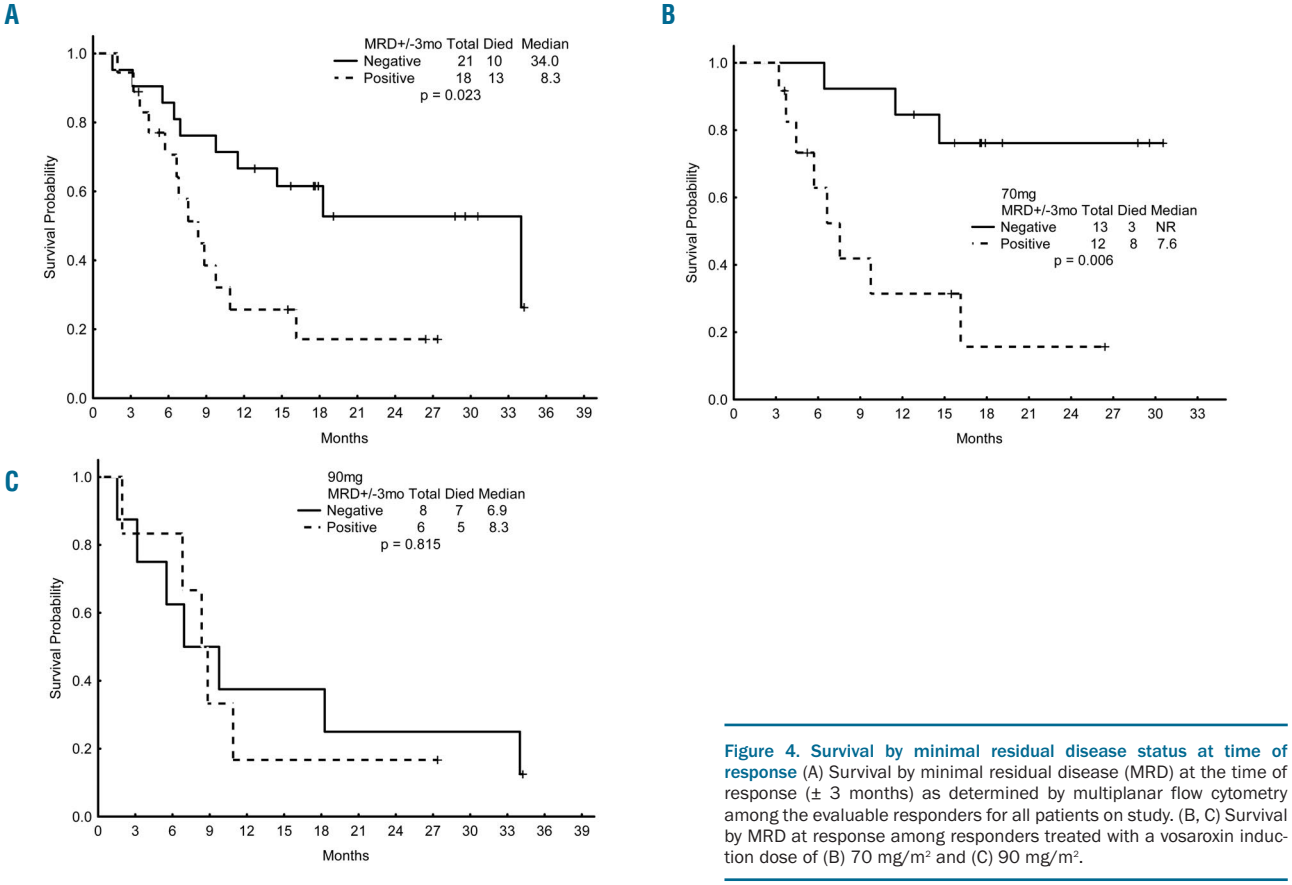

Figure 4. Survival by minimal residual disease status at time of response (A) Survival by minimal residual disease (MRD) at the time of response (± 3 months) as determined by multiplanar flow cytometry among the evaluable responders for all patients on study. (B, C) Survival by MRD at response among responders treated with a vosaroxin induction dose of (B) 70 mg/m^2 and (C) 90 mg/m^2.

further improve outcomes, decitabine was administered at a dose of 20 mg/m^2 in an extended 10-day regimen with a reported CR/ CRi rate of 64%, 8-week mortality of 15%, and median OS of 13.7 months.[26] However, these superior outcomes have not been reproduced. In a randomized trial of azacytidine *versus* standard of care in 488 older patients (age ≥65 years) with AML, a response rate of 27.8% and a median survival of 10.9 months were reported for azacytidine[27] as compared to 25.1% and 6.5 months, respectively for the control arm. The 74% response rate among the patients treated on this trial does, therefore, compare favorably to those achieved with intensive chemotherapy, single-agent decitabine in a 5-day or 10-day dose regimen, or azacytidine in older patients with AML.[4,25-28] Although the median OS of 9.8 months for all patients in this study is similar to that which has been achieved with intensive chemotherapy or HMA, the 70 mg/m^2 dose of vosaroxin at induction produced a clearly improved, encouraging median survival (14.6 months).

The reported incidences of adverse karyotype and *TP53* mutations in older patients with newly diagnosed AML are 20-25% and 5-10%, respectively.[29-32] Typically, patients with these characteristics are resistant to standard cytotoxic therapy, having remission rates of 32-36% and a median survival of 4 – 7 months with standard therapies.[33-35] Welch *et al.* treated 114 patients (88 with AML and 26 with MDS) with a 10-day regimen of decitabine in monthly cycles and reported high rates of morphological remission (46%). They specifically noted higher response rates among patients with an unfavorable cytogenetic profile than among those with intermediate- or favorable-cytogenetic profiles (67% *versus* 37%; $P<0.001$) and among patients with *TP53* mutations as compared to those without *TP53* mutations (100% *versus* 41%; $P<0.001$).[36] The patients in our trial had poor prognostic factors with 35% having an adverse karyotype and 20% having *TP53* mutations. A potential benefit of vosaroxin is its ability to induce apoptosis independently of *TP53* function. In this trial, we noted ORR of 65% and 77% among patients with an adverse karyotype and mutated *TP53*, respectively. In contrast to the findings of Welch *et al.*, the response rates in *TP53* mutated patients were similar to but not better than the ORR of 74% for our entire group. In spite of the improved response rate, the median OS for *TP53* mutated patients was <6 months with the major reason for poor OS among these patients being relapsed/refractory disease. In summary we believe that both our data and those from Welch *et al.* support the use of HMA-based therapies for patients with complex cytogenetic abnormalities and *TP53* mutations, at least to achieve an initial response. However, further approaches including incorporation of novel agents may be needed to significantly improve the survival in this high-risk population.

Three patients had received prior therapy with HMA. Two of these three patients had a response, but their OS was significantly shorter than that of the other patients. In summary, as has been well described in the past, patients who progressed to AML after having received treatment with HMA had a worse outcome than those who had not received prior HMA therapy, although the numbers are small.

MRD status has emerged as a very important prognostic factor for long-term outcomes in AML patients treated with cytotoxic induction.[17,37-39] This was one of the first trials to monitor MRD status and correlate it with outcome in patients treated with hypomethylator-based therapies. A multicolor flow cytometric immunophenotype for detecting MRD in AML was used in this trial. The methodology of MRD detection used at our institution has been previously published.[17,18] MRD at remission was evaluated in 81% of the patients who achieved remission. Half of the patients became MRD negative at the time of achieving remission and achieving MRD negativity was associated with a significantly improved OS.

In conclusion the combination of vosaroxin with decitabine achieves a higher response rate with an equivalent 8-week mortality to that expected with decitabine or azacytidine alone. Patients treated with the 70 mg/m^2 regimen had a median survival of 14.6 months and 51% were alive at 1 year. Prospective randomized trials to compare vosaroxin with decitabine to existing regimens in newly diagnosed older patients with AML are encouraged.

Acknowledgments

Funding support received from Sunesis Pharmaceuticals and the MD Anderson Cancer Centre Leukaemia Support Grant (CCSG) CA016672.

Funding

Sunesis Pharmaceuticals and the MD Anderson Cancer Centre Leukaemia Support Grant (CCSG) CA016672.

Prior presentations: Oral presentation ASH 2014, Oral presentation ASH 2015, Oral presentation EHA 2016.

References

1. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2006. CA Cancer J Clin. 2006;56(2):106-130.
2. Lowenberg B, Downing JR, Burnett A. Acute myeloid leukemia. N Engl J Med. 1999;341(14):1051-1062.
3. Juliusson G, Antunovic P, Derolf A, et al. Age and acute myeloid leukemia: real world data on decision to treat and outcomes from the Swedish Acute Leukemia Registry. Blood. 2009;113(18):4179-4187.
4. Kantarjian H, O'Brien S, Cortes J, et al. Results of intensive chemotherapy in 998 patients age 65 years or older with acute myeloid leukemia or high-risk myelodysplastic syndrome: predictive prognostic models for outcome. Cancer. 2006;106(5): 1090-1098.
5. Appelbaum FR, Gundacker H, Head DR, et al. Age and acute myeloid leukemia. Blood. 2006;107(9):3481-3485.
6. Burnett A, Wetzler M, Lowenberg B. Therapeutic advances in acute myeloid leukemia. J Clin Oncol. 2011;29(5):487-494.
7. Kantarjian HM, Thomas XG, Dmoszynska A, et al. Multicenter, randomized, open-label, phase III trial of decitabine versus patient choice, with physician advice, of either supportive care or low-dose cytarabine for the treatment of older patients with newly diagnosed acute myeloid leukemia. J Clin Oncol. 2012;30(21):2670-2677.
8. Thomas XG, Arthur C, Delaunay J, Jones M, Berrak E, Kantarjian HM. A post hoc sensitivity analysis of survival probabilities in a multinational phase III trial of decitabine in older patients with newly diagnosed acute myeloid leukemia. Clin Lymphoma Myeloma Leuk. 2014;14(1):68-72.
9. Hawtin RE, Stockett DE, Byl JA, et al. Voreloxin is an anticancer quinolone derivative that intercalates DNA and poisons topoisomerase II. PLoS One. 2010;5(4): e10186.
10. Hoch U, Lynch J, Sato Y, et al. Voreloxin, formerly SNS-595, has potent activity against a broad panel of cancer cell lines and in vivo tumor models. Cancer Chemother Pharmacol. 2009;64(1):53-65.
11. Walsby EJ, Coles SJ, Knapper S, Burnett AK.

The topoisomerase II inhibitor voreloxin causes cell cycle arrest and apoptosis in myeloid leukemia cells and acts in synergy with cytarabine. Haematologica. 2011;96(3): 393-399.
12. Stuart RK, Cripe LD, Maris MB, et al. REVEAL-1, a phase 2 dose regimen optimization study of vosaroxin in older poor-risk patients with previously untreated acute myeloid leukaemia. Br J Haematol. 2015;168 (6):796-805.
13. Ravandi F, Ritchie EK, Sayar H, et al. Vosaroxin plus cytarabine versus placebo plus cytarabine in patients with first relapsed or refractory acute myeloid leukaemia (VALOR): a randomised, controlled, double-blind, multinational, phase 3 study. Lancet Oncol. 2015;16(9):1025-1036.
14. Cashen AF, Schiller GJ, O'Donnell MR, DiPersio JF. Multicenter, phase II study of decitabine for the first-line treatment of older patients with acute myeloid leukemia. J Clin Oncol. 2010;28(4):556-561.
15. Willemze R, Suciu S, Archimbaud E, et al. A randomized phase II study on the effects of 5-Aza-2'-deoxycytidine combined with either amsacrine or idarubicin in patients with relapsed acute leukemia: an EORTC Leukemia Cooperative Group phase II study (06893). Leukemia. 1997;11(Suppl 1):S24-27.
16. Schwartsmann G, Fernandes MS, Schaan MD, et al. Decitabine (5-Aza-2'-deoxycytidine; DAC) plus daunorubicin as a first line treatment in patients with acute myeloid leukemia: preliminary observations. Leukemia. 1997;11(Suppl 1):S28-31.
17. Ravandi F, Jorgensen J, Borthakur G, et al. Persistence of minimal residual disease assessed by multiparameter flow cytometry is highly prognostic in younger patients with acute myeloid leukemia. Cancer. 2017;123(3):426-435.
18. Jaso JM, Wang SA, Jorgensen JL, Lin P. Multicolor flow cytometric immunophenotyping for detection of minimal residual disease in AML: past, present and future. Bone Marrow Transplant. 2014;49(9):1129-1138.
19. Luthra R, Patel KP, Reddy NG, et al. Next-generation sequencing-based multigene mutational screening for acute myeloid leukemia using MiSeq: applicability for diagnostics and disease monitoring. Haematologica. 2014;99(3):465-473.
20. Cheson BD, Bennett JM, Kopecky KJ, et al. Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. J Clin Oncol. 2003;21(24):4642-4649.
21. Sievers EL, Larson RA, Stadtmauer EA, et al. Efficacy and safety of gemtuzumab ozogamicin in patients with CD33-positive acute myeloid leukemia in first relapse. J Clin Oncol. 2001;19(13):3244-3254.
22. Thall PF, Simon RM, Estey EH. Bayesian sequential monitoring designs for single-arm clinical trials with multiple outcomes. Stat Med. 1995;14(4):357-379.
23. Kaplan EL, Meier P. Nonparametric-estimation from incomplete observations. J Am Stat Assoc. 1958;53(282):457-481.
24. Buchner T, Berdel WE, Haferlach C, et al. Age-related risk profile and chemotherapy dose response in acute myeloid leukemia: a study by the German Acute Myeloid Leukemia Cooperative Group. J Clin Oncol. 2009;27(1):61-69.
25. Kantarjian H, Ravandi F, O'Brien S, et al. Intensive chemotherapy does not benefit most older patients (age 70 years or older) with acute myeloid leukemia. Blood. 2010;116(22):4422-4429.
26. Blum W, Garzon R, Klisovic RB, et al. Clinical response and miR-29b predictive significance in older AML patients treated with a 10-day schedule of decitabine. Proc Natl Acad Sci USA. 2010;107(16):7473-7478.
27. Dombret H, Seymour JF, Butrym A, et al. International phase 3 study of azacitidine vs conventional care regimens in older patients with newly diagnosed AML with >30% blasts. Blood. 2015;126(3):291-299.
28. Tallman MS, Gilliland DG, Rowe JM. Drug therapy for acute myeloid leukemia. Blood. 2005;106(4):1154-1163.
29. Grimwade D, Walker H, Harrison G, et al. The predictive value of hierarchical cytogenetic classification in older adults with acute myeloid leukemia (AML): analysis of 1065 patients entered into the United Kingdom Medical Research Council AML11 trial. Blood. 2001;98(5):1312-1320.
30. Stirewalt DL, Kopecky KJ, Meshinchi S, et al. FLT3, RAS, and TP53 mutations in elderly patients with acute myeloid leukemia. Blood. 2001;97(11):3589-3595.
31. Schoch C, Kern W, Schnittger S, Buchner T, Hiddemann W, Haferlach T. The influence of age on prognosis of de novo acute myeloid leukemia differs according to cytogenetic subgroups. Haematologica. 20040;89(9):1082-1090.
32. Rucker FG, Schlenk RF, Bullinger L, et al. TP53 alterations in acute myeloid leukemia with complex karyotype correlate with specific copy number alterations, monosomal karyotype, and dismal outcome. Blood. 2012;119(9):2114-2121.
33. Nahi H, Lehmann S, Bengtzen S, et al. Chromosomal aberrations in 17p predict in vitro drug resistance and short overall survival in acute myeloid leukemia. Leuk Lymphoma. 2008;49(3):508-516.
34. Seifert H, Mohr B, Thiede C, et al. The prognostic impact of 17p (p53) deletion in 2272 adults with acute myeloid leukemia. Leukemia. 2009;23(4):656-663.
35. Kadia TM, Jain P, Ravandi F, et al. TP53 mutations in newly diagnosed acute myeloid leukemia: clinicomolecular characteristics, response to therapy, and outcomes. Cancer. 2016 Jul 26. [Epub ahead of Print].
36. Welch JS, Petti AA, Miller CA, et al. TP53 and decitabine in acute myeloid leukemia and myelodysplastic syndromes. N Engl J Med. 2016;375(21):2023-2036.
37. Freeman SD, Virgo P, Couzens S, et al. Prognostic relevance of treatment response measured by flow cytometric residual disease detection in older patients with acute myeloid leukemia. J Clin Oncol. 2013;31 (32):4123-4131.
38. Ouyang J, Goswami M, Tang G, et al. The clinical significance of negative flow cytometry immunophenotypic results in a morphologically scored positive bone marrow in patients following treatment for acute myeloid leukemia. Am J Hematol. 2015;90 (6):504-510.
39. Chen X, Xie H, Wood BL, et al. Relation of clinical response and minimal residual disease and their prognostic impact on outcome in acute myeloid leukemia. J Clin Oncol. 2015;33(11):1258-1264.