

A phase 1b/2 study of vosaroxin in combination with cytarabine in patients with relapsed or refractory acute myeloid leukemia

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Methods

Inclusion and exclusion criteria

Phase 1B inclusion criteria:

- ≥ 18 years of age
- Relapsed or refractory acute myeloid leukemia (AML) subtypes defined by the World Health Organization (WHO), except acute promyelocytic leukemia, for which no standard therapies are expected to result in a durable remission. Relapsed or refractory disease may be de novo AML or secondary AML (from an antecedent hematologic disorder such as myelodysplastic syndrome [MDS] or therapy-related AML [t-AML])
- Treated with 1 to 3 induction/reinduction AML regimens; prior induction or consolidation therapy with cytarabine allowed
- $\geq 10\%$ blasts by bone marrow biopsy or aspirate
- Eastern Cooperative Oncology Group (ECOG) performance status score of 0 or 1

Phase 2 inclusion criteria:

- Relapsed or refractory AML subtypes defined by the WHO, except acute promyelocytic leukemia. Relapsed or refractory disease may be de novo AML or secondary AML (from an antecedent hematologic disorder such as MDS or t-AML). For refractory AML, no standard therapies are expected to result in durable remission.
- Relapsed AML: Treated with ≤ 1 induction/reinduction AML regimen that resulted in a complete remission (CR) of ≥ 3 months duration (i.e. patients in first relapse). Prior induction/reinduction or

consolidation therapy with cytarabine is allowed. One biologic or noncytotoxic therapy after relapse is allowed. Maintenance regimen of hypomethylating agents or other biologic agents following CR and consolidation is allowed.

- Refractory AML: Primary refractory disease, defined as no CR following initial intensive therapy (≤ 2 cycles) and ≥ 1 cycle had to be an anthracycline/anthracenedione and cytarabine-based therapy

Exclusion criteria (all patients):

- Allogeneic BM transplant/stem cell transplant. Autologous BM or stem cell transplantation is allowed if it was part of a consolidation regimen and was completed at least 3 months prior (unless hypocellular) to the first treatment with vosaroxin injection
- Persistent, clinically significant, chronic toxicities from prior AML therapy that would contraindicate the patient's participation in the clinical study due to safety concerns or compliance with study procedures
- Acute promyelocytic leukemia
- Disseminated intravascular coagulation
- Active infections, unless adequately treated with antibiotic, antiviral, or antifungal agents within 7 days before induction day 1; prophylactic antibiotics are acceptable
- Active CNS involvement by AML
- Other active malignancies or other malignancies within the last 12 months except nonmelanoma skin cancer or cervical intraepithelial neoplasia
- Patients requiring hemodialysis or peritoneal dialysis
- History of myocardial infarction within the 3 months before treatment with vosaroxin injection
- History of cerebrovascular accident/transient ischemic attack (CVA/TIA) within the 3 months before treatment with vosaroxin injection

- Thromboembolic event (deep vein thrombosis or pulmonary embolus) within 28 days before treatment with vosaroxin injection
- Investigational products within 28 days before treatment with vosaroxin injection, and noninvestigational cancer therapies or radiation therapy within 14 days before treatment with vosaroxin injection, with the exception of hydroxyurea; discontinue hydroxyurea at least 24 hours before the first treatment with vosaroxin injection
- Known intolerance to cytarabine or known allergy to D-sorbitol or methanesulfonic acid (excipients used in vosaroxin injection)
- Prior exposure to vosaroxin injection
- Women who are pregnant or breastfeeding
- Women of childbearing potential or male patients who have partners of childbearing potential unwilling to use an approved, effective means of contraception according to the study site's standards
- Any other medical, psychological, or social condition that would contraindicate the patient's participation in the clinical study due to safety concerns or compliance with study procedures, in the opinion of the Principal Investigator (PI), Medical Monitor, or designee (Medical Monitor)

Study treatment

Patients were enrolled at 7 US clinical sites and assigned in successive cohorts to receive escalating doses of vosaroxin (given as a 10-minute infusion on days 1 and 4) in combination with cytarabine on 1 of 2 schedules (A and B). Based on findings from nonclinical studies of the vosaroxin plus cytarabine combination showing synergy, vosaroxin (starting dose 10 mg/m²) was given in combination with a 24-hour continuous intravenous (IV) infusion of cytarabine (400 mg/m²/day, days 1-5) in Schedule A. In schedule B, vosaroxin (starting dose 70 mg/m²) was given in combination with a 2-hour IV infusion of

cytarabine (1 g/m²/day, days 1-5). This dosing schedule would maintain plasma vosaroxin levels at ≥1 μM, the in vitro IC₉₀, for an adequate period, and balance efficacy and safety considerations with an intermediate dose of cytarabine. Up to 2 induction cycles were administered (induction and reinduction cycles). Patients with stable disease or a reduction in BM blasts and no persistent clinically significant toxicity after induction were eligible for reinduction, to be initiated no earlier than induction day 15 and no later than induction day 57. Patients with CR or CR with incomplete blood count recovery (CRi) and an absolute neutrophil count (ANC) ≥500 cells/μL were eligible for 1-2 cycles of consolidation therapy consisting of the same treatment received in induction. Prophylactic antibiotics, antifungal agents, and antiviral agents were administered per individual institutional guidelines. Approximately 15 patients in first relapse were enrolled at the maximum tolerated dose (MTD) of each schedule. Up to 25 additional patients with primary-refractory disease were enrolled at the MTD for schedule B.

Evaluation of dose-limiting toxicity (DLT)

In the dose-escalation phase, patients were enrolled in cohorts of 3. If 1 patient experienced DLT, 3 additional patients were enrolled at that dose level. If 0/3 patients or 1/6 experienced DLT, enrollment proceeded at the next dose level. If ≥ 2 patients experienced DLT, the immediate preceding dose was expanded to 6 patients. Additional patients were enrolled if patients proved nonevaluable (e.g. did not receive all doses or complete evaluation for DLT). Grade 4 neutropenia or thrombocytopenia lasting > 8 weeks without residual leukemia were considered DLT. Clinically significant and treatment-related grade ≥ 3 nonhematologic AEs were also considered DLT *except* for the following: nausea, vomiting, or diarrhea controlled with antiemetic/antidiarrheal therapy, alopecia, grade 3 mucositis for < 7 days, grade 3 liver enzyme elevation for < 7 days, or infection/febrile neutropenia controlled by antibiotics.

Safety assessments

AEs were coded according to the Medical Dictionary for Regulatory Activities (v 10.0). Serious AEs (SAEs) were those deemed immediately life-threatening or that resulted in death, hospitalization, persistent or significant disability, congenital anomaly, or medical or surgical intervention to preclude permanent impairment. BM biopsy or aspirate was performed at screening, on day 15 of induction and reinduction cycles, at hematologic recovery, and as clinically indicated.

Analysis of pharmacokinetic parameters

For PK studies, blood samples were collected predose; at the end of the vosaroxin infusion; and at 2, 5, 8, 24, 48, and 72 hours postinfusion for the first and second vosaroxin doses in cycle 1. Urine samples were obtained predose and during the 24-hour postdose period on day 1 of cycle 1 for patients enrolled in the 80 mg/m² dose cohort in schedule A and the 90 mg/m² cohort in schedule B. Bioanalytical assays for vosaroxin (plasma and urine) and vosaroxin metabolites (urine) were performed by Alta Analytical Laboratory, Inc (El Dorado Hills, CA) using validated liquid chromatography-tandem mass spectrometry methods.¹ Standard PK parameters (area under the concentration versus time curve [AUC], clearance, half-life, mean residence time, and volume of distribution at steady state) were derived from plasma concentration–time data, and dose proportionality and drug accumulation were assessed. Dose proportionality was analyzed by a power model, $AUC_{inf} = \alpha(\text{dose})^\beta$ where AUC_{inf} and dose (mg) are from day 1 for all patients in schedule A and schedule B; α is the intercept and β is the slope on a log-log scale. The effects of age, body weight, and body surface area on vosaroxin clearance (day 1) were evaluated using linear regression. Clearance (day 1) was compared between males and females, and between patients < 65 years and \geq 65 years of age using 2-sample *t* tests. Drug accumulation was calculated as a ratio of vosaroxin exposure, AUC_{72d4}/AUC_{72d1} , and analyzed using paired *t* tests. Vosaroxin clearance values were log transformed for all statistical analyses. Renal excretion of vosaroxin and N-desmethyl-

vosaroxin was estimated by the amount excreted in 24 hours per vosaroxin dose.

Analysis of pharmacodynamic parameters

Biomarkers for DNA damage response to treatment with vosaroxin or cytarabine were identified in K562 cells and then evaluated in primary AML peripheral blood samples; pDNA-PKcs and pCHK2, both markers of DNA double-strand breaks,² were selected for evaluation of clinical samples. To assess induction of a mechanism-based DNA damage response to treatment, blood samples were collected from patients predose and 2, 5, 24, and 48 hours postdose using Cell Preparation Tubes™ (Becton Dickinson & Company, Franklin Lakes, NJ) containing sodium heparin. Peripheral blood mononuclear cells (PBMCs) were isolated at the clinical sites, shipped frozen to ACM (Rochester, NY) for central storage, and sent to Sunesis Pharmaceuticals for analysis upon request. PBMCs were lysed, and levels of pDNA-PKcs and pCHK2 were investigated by Western blot analysis. Patients with increased levels of pDNA-PKcs and pCHK2 were defined as PD responders.

Study endpoints

In the dose-escalation phase, the primary endpoint was to assess DLT and determine the MTD for each schedule. In the expansion phase, the primary endpoint was the CR rate (CR + CRi); secondary endpoints were leukemia-free survival (LFS), overall survival (OS), and safety. Vosaroxin PK profile and pharmacodynamic (PD) activity were also characterized.

Statistical analyses

Approximately 15 patients in first relapse were enrolled at the MTD of each schedule. Up to 25 additional patients with primary-refractory disease were enrolled at the MTD for schedule B.

Data were summarized using descriptive statistics. Where appropriate, 95% confidence intervals (CIs) are

presented. For time-to-event endpoints (LFS and OS), point estimates of the median duration were calculated along with 2-sided 95% CIs using Kaplan-Meier methods. LFS was defined as the time between the date of CR and the date of relapse or death from any cause, whichever occurred first. For calculation of LFS, patients were not censored for subsequent therapies such as maintenance therapy or transplant. OS was defined as the time between the date of first study treatment and the date of death from any cause. For calculation of OS, patients were censored at the time of the last date confirmed alive.

References

1. Lancet JE, Ravandi F, Ricklis RM, Cripe LD, Kantarjian HM, Giles FJ, et al. A phase Ib study of vosaroxin, an anticancer quinolone derivative, in patients with relapsed or refractory acute leukemia. *Leukemia*. 2011;25(12):1808-1814.
2. Summers KC, Shen F, Sierra Potchanant EA, Phipps EA, Hickey RJ, Malkas LH. Phosphorylation: the molecular switch of double-strand break repair. *Int J Proteomics*. 2011;2011:373816.

Supplementary Table 1. Dose escalation of vosaroxin in combination with cytarabine in acute myeloid leukemia patients and incidence of dose-limiting toxicity (DLT)

Schedule	Vosaroxin dose level, mg/m ²	Patients treated, n	DLT, n	Description of DLT
A	10	4	0	
	20	3	0	
	34	4	0	
	50	6	0	
	70	7	1	Grade 5 sepsis
	80	8	1	Grade 3 stomatitis (oral mucositis)
	90	7	2	Grade 3 bowel obstruction; grade 3 stomatitis (oral mucositis) lasting > 7 days
B	70	6	0	
	80	6	1	Grade 3 odynophagia
	90	6	1	Grade 3 stomatitis (oral mucositis)/esophagitis lasting > 7 days

Supplementary Table 2. Pharmacokinetic parameters by schedule, dosing day, and dose cohort

Day	Schedule*	Dose cohort, mg/m ²	n	Pharmacokinetic parameter									
				AUC _∞ , h·ng/mL		CL, L/h		t _{1/2} , h		MRT _∞ , h		V _{ss} , L	
				Mean	CV%	Mean	CV%	Mean	CV%	Mean	CV%	Mean	CV%
1	A	10-90	54	NA	NA	4.08	35	24.57	38	32.59	42	122.7	32
	B	70-90	44	NA	NA	4.17	29	23.32	25	30.86	29	123.27	31
	B	90 [†]	32	47612.29	29	4.05	31	23.58	25	31.31	30	122.05	35
4 [‡]	A	10-90	54	NA	NA	3.91	32	23.71	44	30.47	40	112.85	36
	B	70-90	43	NA	NA	4.09	30	21.05	31	27.68	31	107.89	31
	B	90 [†]	31	55198.15	36	4.02	32	22	31	29.02	31	111.35	33

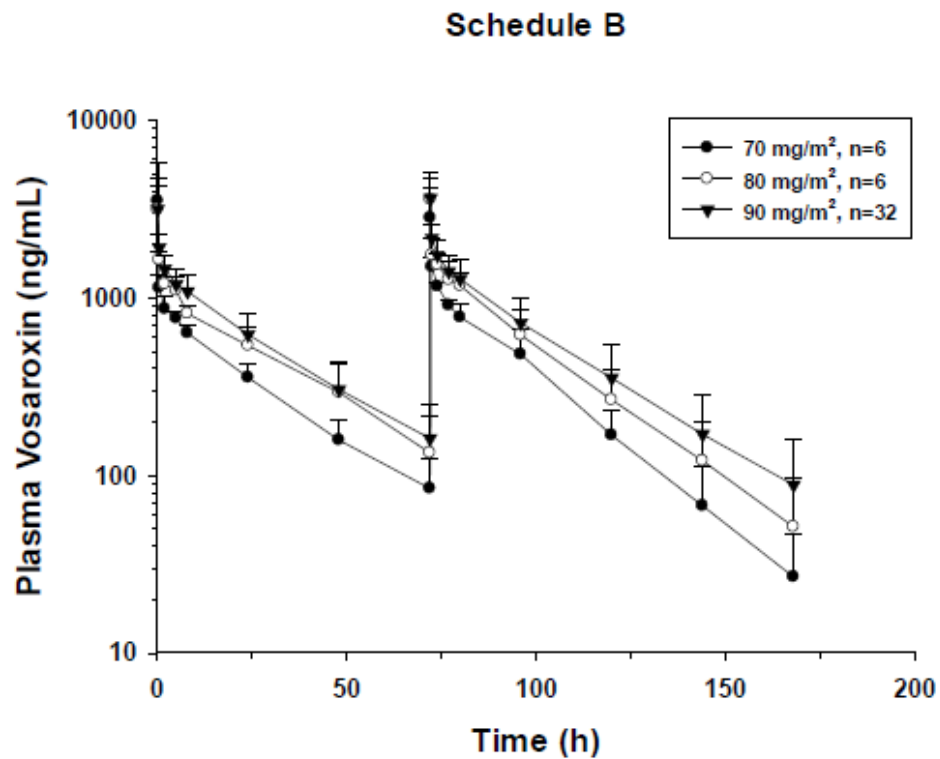
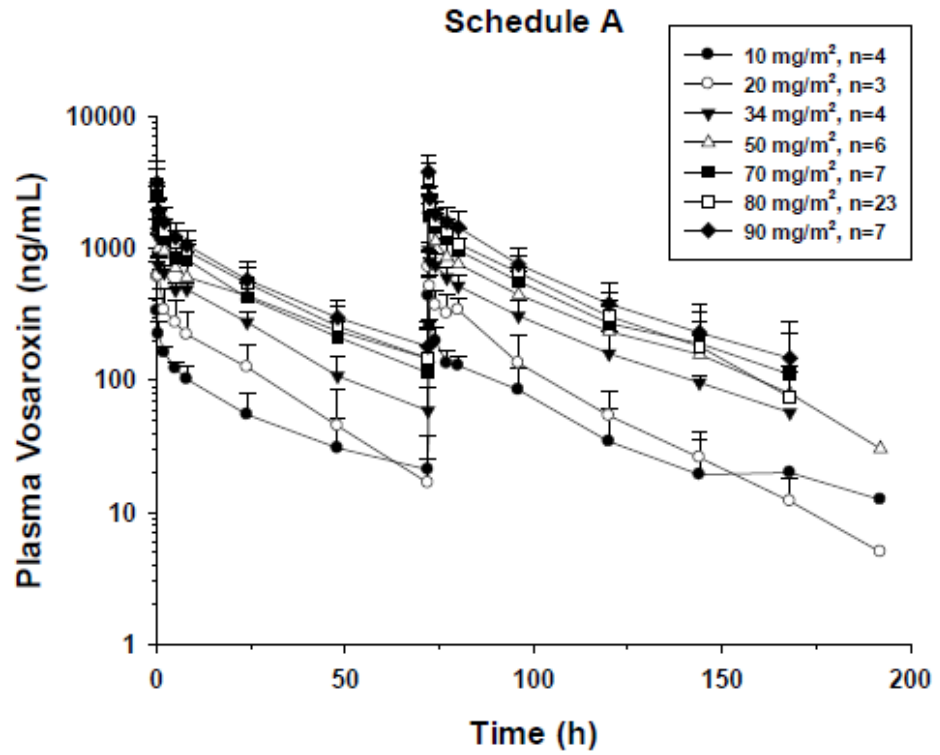
*Schedule A: vosaroxin (days 1, 4) and cytarabine (24-h infusion on days 1-5) in a 28-day cycle. Schedule B: vosaroxin (days 1, 4) and cytarabine (2-h infusion on days 1-5) in a 28-day cycle.

[†]Maximum tolerated dose.

[‡]Pharmacokinetic analysis by a steady-state approach, where CL = CL_{ss}.

AUC_∞, area under the plasma concentration–time curve from time zero to infinity; CL, clearance; CV, coefficient of variation; MRT_∞, mean residence time extrapolated to infinity; t_{1/2}, half-life; V_{ss}, volume of distribution at steady state.

Supplementary Figure 1. Mean plasma concentration of vosaroxin in acute myeloid leukemia patient over time, by schedule and dose cohort (cycle 1).



Supplementary Figure 2. Vosaroxin-induced pharmacodynamic response in K562 cells. Cells were treated with a dose titration of vosaroxin (0.3-9 μ M), 0.1 μ M doxorubicin, or vehicle alone, and samples were taken at 2, 4, 6, and 24 hours for analysis. Actin served as a loading control.

