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REVIEW ARTICLE

Molecular and Pharmacologic Properties of the Anticancer Quinolone Derivative Vosaroxin: A New Therapeutic Agent for Acute Myeloid Leukemia

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Abstract Vosaroxin is a first-in-class anticancer quinolone derivative that targets topoisomerase II and induces site-selective double-strand breaks in DNA, leading to tumor cell apoptosis. Vosaroxin has chemical and pharmacologic characteristics distinct from other topoisomerase II inhibitors due to its quinolone scaffold. The efficacy and safety of vosaroxin in combination with cytarabine were evaluated in patients with relapsed/refractory acute myeloid leukemia (AML) in a phase III, randomized, multicenter, double-blind, placebo-controlled study (VALOR). In this study, the addition of vosaroxin produced a 1.4-month improvement in median overall survival (OS; 7.5 months with vosaroxin/cytarabine vs. 6.1 months with placebo/cytarabine; hazard ratio [HR] 0.87, 95 % confidence interval [CI] 0.73−1.02; unstratified log-rank $p = 0.061$; stratified log-rank $p = 0.024$), with the greatest OS benefit observed in patients ≥60 years of age (7.1 vs. 5.0 months; HR 0.75, 95 % CI 0.62−0.92; $p = 0.003$) and patients with early relapse (6.7 vs. 5.2 months; HR 0.77, 95 % CI 0.59−1.00; $p = 0.039$), two AML patient groups that typically have poor prognosis. Here we review the chemical and pharmacologic properties of vosaroxin, how these properties are distinct from those of currently available topoisomerase II inhibitors, how they may contribute to the efficacy and safety profile observed in the VALOR trial, and the status of clinical development of vosaroxin for treatment of AML.

Key Points

Vosaroxin is a first-in-class anticancer quinolone derivative that inhibits topoisomerase II causing tumor cell apoptosis.

Due to the stability of its quinolone core, vosaroxin is not associated with significant formation of toxic metabolites, free radicals, or reactive oxygen species, which are associated with off-target organ damage and cardiotoxicity.

Vosaroxin is not a substrate for the P-glycoprotein efflux pump, and vosaroxin activity is maintained in cells with p53 deletion thus evading two common mechanisms of drug resistance.

In the phase III VALOR trial, the addition of vosaroxin to cytarabine was shown to provide clinical benefit to some patients with relapsed or refractory AML, particularly older patients and those with early relapsed disease.

The unique chemical and pharmacologic characteristics of vosaroxin may contribute to the efficacy and safety profile observed in the phase III VALOR trial.

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1 Introduction

Vosaroxin is an anticancer quinolone derivative (AQD) in development for patients with relapsed/refractory acute myeloid leukemia (R/R AML). It is the first in a novel class of antineoplastic agents (non-antibacterial fluoroquinolone derivatives) recognized by the United States Adopted Names Council [1]. Vosaroxin induces replication-dependent DNA damage by intercalating DNA and inhibiting topoisomerase II, which induces cancer cell apoptosis [2]. This review describes the chemical and pharmacologic properties of vosaroxin, highlights the differences as compared with currently approved topoisomerase II inhibitors, and summarizes the clinical development of vosaroxin for AML.

2 Vosaroxin Discovery

Nalidixic acid was the first quinolone synthesized, in the early 1960s, demonstrating antibacterial properties [3]. Antibacterial quinolones induce DNA damage by inhibiting bacterial topoisomerases, DNA gyrase and topoisomerase IV, which are functional analogs of mammalian topoisomerase II [4, 5]. The homology between mammalian and bacterial topoisomerases, and the fact that mammalian topoisomerase II is a well-established target of antineoplastic drugs [6–9], provided the rationale for screening and identification of AQDs that selectively target mammalian topoisomerase II [6–11]. Although eukaryotic DNA topoisomerase II and bacterial homologs share regions with >50 % amino acid sequence homology and a conserved three-domain tertiary structure, there are substantial differences in the enzymatic reaction mechanism and quaternary structure of the eukaryotic and bacterial enzymes [12, 13]. These differences may underlie the specificity demonstrated by antibiotics that are potent inhibitors of bacterial topoisomerases but are effective only at very high, clinically irrelevant concentrations against the eukaryotic homologs. Conversely, this distinction allows selection of inhibitors specific for human DNA topoisomerase II [14].

Vosaroxin (SNS-595, voreloxin) was selected from a mouse P388 leukemia cell-based screen that examined structure-activity relationships of novel quinolone derivatives to identify a potent antineoplastic agent that preferentially targets mammalian topoisomerase II [11]. The selectivity of vosaroxin for mammalian topoisomerase II was substantiated by the absence of antimicrobial activity in vitro against *Candida albicans*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* at vosaroxin concentrations approximately 20-fold higher than the average maximum clinical concentration (data on file, Sunesis Pharmaceuticals, South San Francisco, CA, USA).

3 Interaction of Vosaroxin with DNA and the Topoisomerase II Cleavage Complex

Topoisomerase II is essential for the survival of eukaryotic cells [8, 9, 12, 15, 16]. The enzyme maintains DNA topology throughout replication, supporting correct chromosome condensation, decondensation, and segregation. Topoisomerase II performs these functions via a choreographed sequential decatenation/concatenation of the DNA helix, catalyzing formation of a double-strand break in DNA and passage of an intact DNA strand through the cleavage site; the enzymatic sequence is completed by religation of the double-strand DNA break [6–9, 15, 16]. Inhibitors of topoisomerase II classified as "topoisomerase poisons" act by stabilizing the covalent topoisomerase II/DNA complex (cleavage complex) after the DNA has been cleaved. This results in the conversion of transient DNA double-strand breaks into permanent lesions and subsequently causes cell-cycle arrest and apoptosis in replicating cells [7, 8]. Examples of topoisomerase II poisons are the anthracyclines doxorubicin, daunorubicin, and idarubicin, the anthracenedione mitoxantrone, and the epipodophyllotoxin etoposide.

Both topoisomerase II poisoning and DNA intercalation contribute to vosaroxin activity [2]. Vosaroxin acts as a topoisomerase II poison, stabilizing cleavage complexes formed by topoisomerase II α and II β isoforms, and resulting in an accumulation of DNA double-strand breaks, perhaps via prevention of DNA ligation [2, 17]. Similar to doxorubicin, daunorubicin, idarubicin, and mitoxantrone (and unlike etoposide), vosaroxin directly intercalates DNA [6–8, 18]. As with other intercalating topoisomerase II inhibitors, higher levels of DNA breaks are seen at lower vosaroxin concentrations; at concentrations above 1.0 μM vosaroxin, a decrease in DNA breaks is observed [2, 14]; this finding may be due to catalytic inhibition or restricted access of topoisomerase II as intercalation into DNA increases. Notably, etoposide, mitoxantrone, and the anthracyclines also exhibit non-topoisomerase II-dependent DNA damage due to metabolic activation and oxidative stress [19–27]. In contrast, these mechanisms do not contribute significantly to vosaroxin activity, which appears to be mediated exclusively through DNA intercalation and topoisomerase II inhibition [2, 31, 37].

Vosaroxin targets actively replicating cells; the extent of DNA damage is cell-cycle dependent, with dose-dependent damage observed mainly in late G2/M and S cell-cycle phases [17]. This DNA damage induces G2/M arrest and S-phase lag [2, 17, 28]. Maximum generation of double-

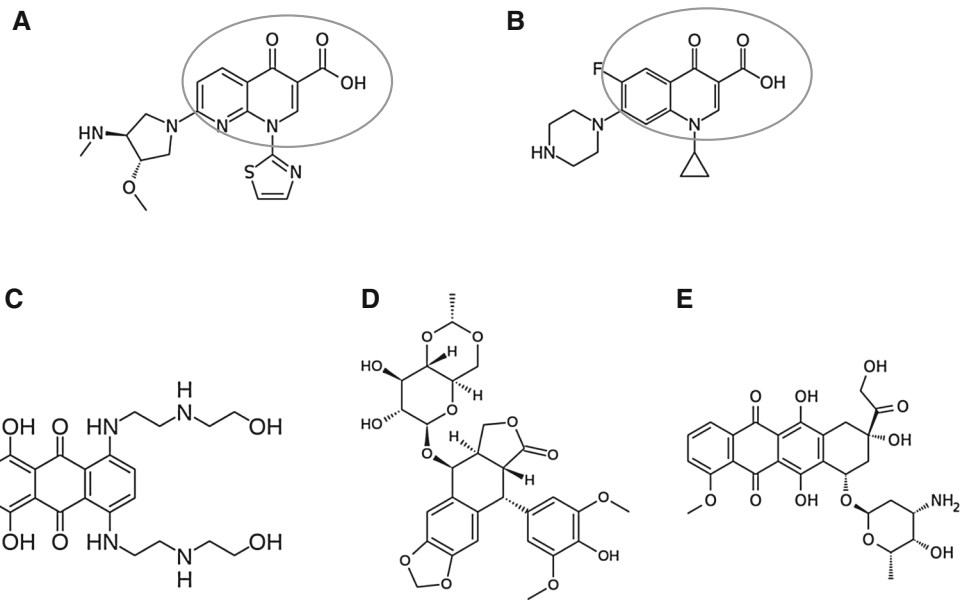

Fig. 1 Distinct chemical structures of topoisomerase II inhibitors. The quinolone core is circled on vosaroxin (**a**) and ciprofloxacin (**b**) to emphasize the differences from other classes of topoisomerase II inhibitors: **c** anthracenedione (mitoxantrone); **d** epipodophyllotoxin (etoposide); and **e** anthracycline (doxorubicin)

strand breaks and cytotoxic activity occurs in G2/M for both vosaroxin and the anthracyclines, consistent with peak topoisomerase II expression at G2 [17]. Conversely, vosaroxin induces a different DNA damage pattern in S-phase as compared with the anthracycline doxorubicin. Unlike with doxorubicin, double-strand breaks were not detected in S-phase with vosaroxin. Vosaroxin appears to prolong S-phase possibly due to torsional stress from cleavage complexes near sites of DNA replication that cause the replication fork to stall [17].

Topoisomerase II is a metalloenzyme with two metal ion-binding sites coordinated through protein catalytic pockets. The catalytic activity of topoisomerase II is mediated by magnesium ions (Mg^{2+}), which facilitate DNA bending and subsequent cleavage [29]. Quinolones and quinolone derivatives also act as sequestering ligands, with binding sites that can chelate divalent metal cations such as Mg^{2+} in 1:1 or 1:2 (metal:quinolone) stoichiometry. X-ray crystallography has revealed that two Mg^{2+} ions mediate the interaction between quinolones and topoisomerase IV [30], and coordination of Mg^{2+} has been shown to play a critical role in quinolone-based molecule activity [30–32]. In vitro studies show that, unlike with the anthracyclines (14), Mg^{2+} coordination is required for vosaroxin activity, similar to its quinolone predecessors [2].

The functional differences observed between vosaroxin and classic topoisomerase II inhibitors result from the unique vosaroxin scaffold. The resulting three-dimensional structure of vosaroxin is distinct from those of other topoisomerase II inhibitors; quinolones have a "wedge" shape, in contrast to the planar form of anthracyclines, supporting a mechanistically distinct interaction with DNA (Fig. 1) [30, 33, 34]. Vosaroxin causes site-selective DNA damage in G/C-rich sequences [2], which is characteristic of quinolone-induced DNA damage. In contrast, anthracyclines favor 3′ A at the cleavage site [2, 35, 36].

4 The Quinolone Scaffold of Vosaroxin is Chemically Stable and Minimally Metabolized

Vosaroxin's quinolone scaffold confers chemical and pharmacologic characteristics distinct from classic topoisomerase II poisons used in AML treatment. Compared with currently approved topoisomerase II inhibitors, vosaroxin is minimally metabolized because of its stable quinolone core. In vitro studies in human microsomes demonstrated that >97 % of vosaroxin remained unchanged after incubating for up to 60 min [37]. Consistent with in vitro data, unchanged vosaroxin was the major species identified in plasma, urine, and bile following intravenous (IV) administration of [^{14}C]-vosaroxin to rats [37]. *N*-Desmethylvosaroxin, an equipotent metabolite of vosaroxin, was the sole metabolite (M4) identified in the plasma of rats, monkeys, and humans, accounting for ≤3 % of the total vosaroxin exposure (data on file, Sunesis Pharmaceuticals, South San Francisco, CA, USA) [37].

Vosaroxin is not associated with significant formation of free radicals or reactive oxygen species (ROS) [2, 37],

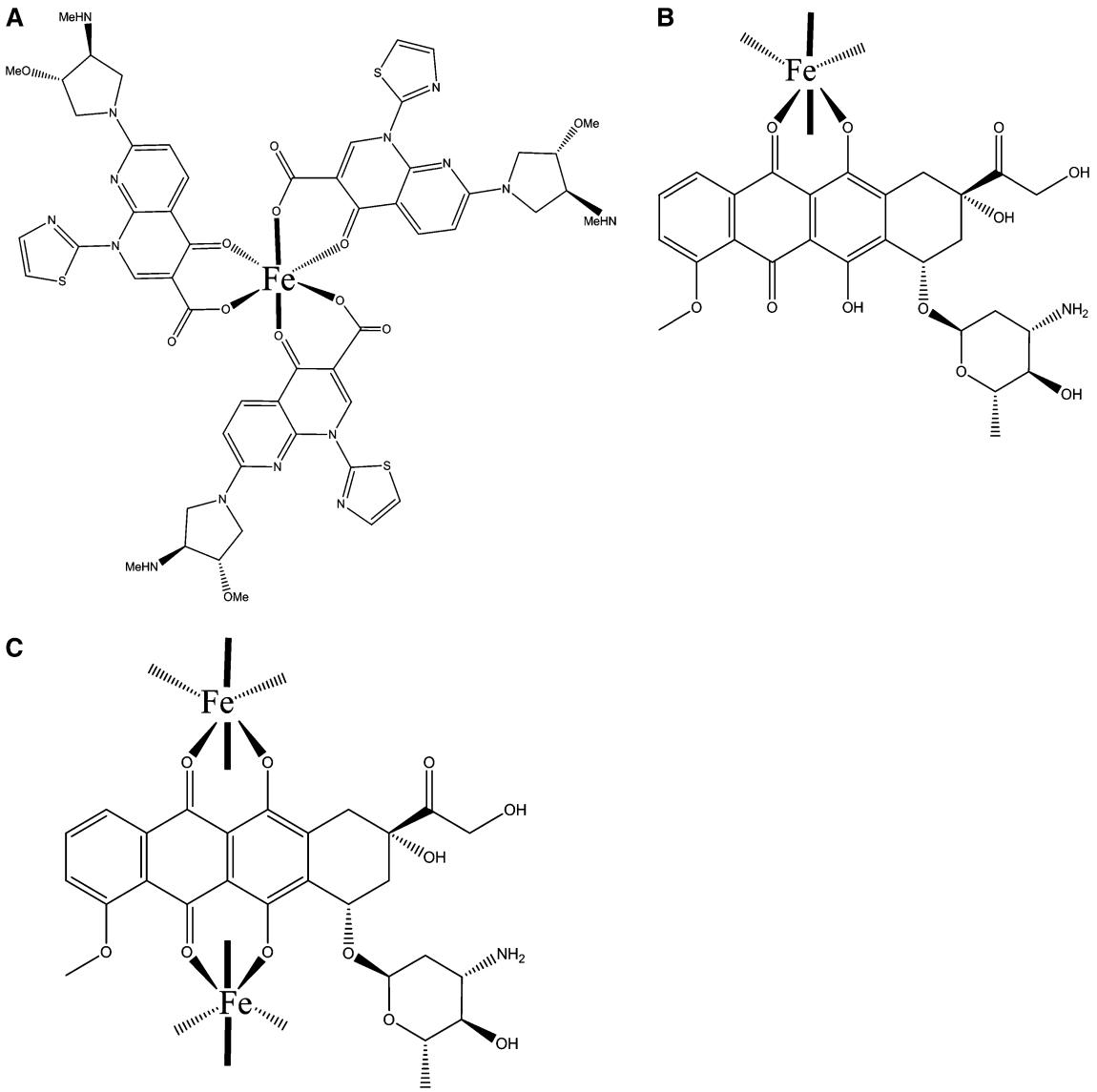

Fig. 2 Fe^{3+} complexes formed by vosaroxin and doxorubicin. **a** 1:3 (Fe^{3+}:vosaroxin) complex, **b** 1:1, and **c** 2:1 (Fe^{3+}:doxorubicin) complex Images are based on Kara et al. 1991 [41] and Drechsel et al. 2001 [42]

whereas anthracyclines mediate formation of ROS in the forms of hydrogen peroxide (H_2O_2) and superoxide radical anion (O_2^-) via multiple mechanisms. One well-characterized mechanism of anthracycline-mediated ROS formation involves Fe^{3+} complexation and redox cycling [31, 38–40]. The interaction with endogenous metal ions, including magnesium and iron, is fundamental to the mechanism of action of quinolone- and anthracycline-based topoisomerase II inhibitors. Iron complexes can lead to ROS formation via a trivalent, Fe^{3+} complexation. Vosaroxin and doxorubicin bind Fe^{3+} with comparable strength; however, vosaroxin forms a stable complex with Fe^{3+} at a 1:3 (metal:vosaroxin) stoichiometry [$Fe(vosaroxin)_3$], where all the reactive sites on Fe^{3+} are occupied, whereas doxorubicin binds at a 1:1 and 2:1 (metal:doxorubicin) stoichiometry, leaving exposed iron-reactive sites (Fig. 2) [31, 41, 42]. At physiologic pH, doxorubicin forms a mixture of labile protonated ligand species; in contrast, vosaroxin predominantly exists as [$Fe(vosaroxin)_3$], a more thermodynamically stable species where the Fe^{3+} ion is coordinated to six O-atoms of the three vosaroxin ligands, leaving no unoccupied iron orbital (Fig. 3) [31]. Therefore, the vosaroxin-iron complexes do not support production of ROS, because the fully occupied iron coordination geometry does not permit free radical formation. This iron coordination geometry and the minimal metabolism of vosaroxin are consistent with experiments

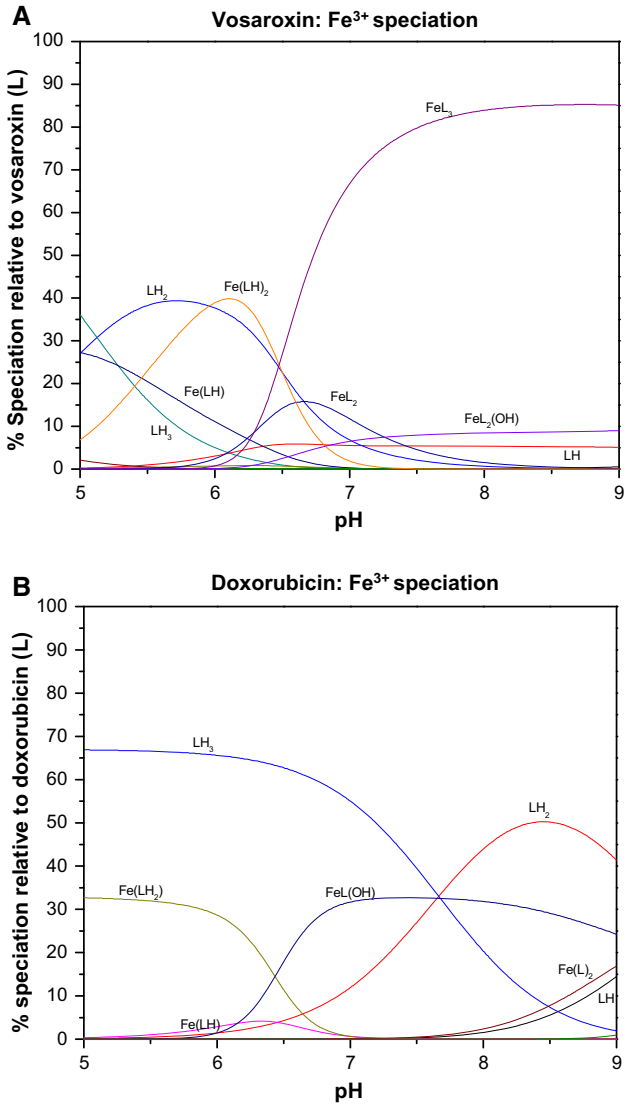

Fig. 3 Speciation plots for solutions of vosaroxin (**a**) or doxorubicin (**b**) with Fe^{3+} as a function of pH. At physiological pH 7.4 (*vertical line*), the predominant Fe^{3+}:vosaroxin species is one Fe^{3+} coordinated by three vosaroxin ligands (FeL_3). For doxorubicin, the predominant species at pH 7.4 is the noncoordinated, charged doxorubicin ligand (LH_3) [31]

in colorectal cancer cells showing limited ROS with vosaroxin versus substantial ROS with doxorubicin [2].

Anthracyclines concentrate in the mitochondria of cardiac cells, where the production of ROS and other toxic metabolites has been implicated in cumulative cardiotoxicity [38–40, 43]. The minimal formation of ROS and other toxic metabolites may limit vosaroxin off-target cardiotoxicity. In the placebo-controlled VALOR study, there was no significant difference in cardiac adverse events (AEs) between patients treated with vosaroxin plus cytarabine and those treated with cytarabine alone [44]. Vosaroxin may be a viable alternative for AML patients at risk of anthracycline-mediated cardiotoxicity because of prior exposure or co-morbidities.

5 Preclinical Evidence of Vosaroxin Antineoplastic Activity

Vosaroxin exhibits potent in vitro activity in cancer cell lines from diverse tissue origins. In 19 solid tumor and hematologic cancer cell lines, the mean half-maximal inhibitory concentration was 345 nM (range 40−1155 nM) [33, 45]. Vosaroxin demonstrated comparable or greater in vivo cytotoxicity compared with etoposide, doxorubicin, irinotecan, cisplatin, paclitaxel, and 5-fluorouracil in a wide range of human tumors (Table 1). In hematologic cancer models, vosaroxin demonstrated increased tumor growth inhibition compared with etoposide and doxorubicin. Notably, radiolabeling experiments in mice indicate that vosaroxin crosses the blood–brain barrier (manuscript in preparation); although brain tissues showed that tissue:plasma ratios were $\leq$1.5, levels of radioactivity in brain indicated the presence of vosaroxin at concentrations associated with anticancer activity in vitro [33, 45]. In contrast, anthracyclines and the anthracenedione mitoxantrone do not cross the blood–brain barrier [46, 47].

Drug efflux by P-glycoprotein 1 efflux transporter (P-gp) is a common drug-resistance pathway in human cancers; unlike etoposide and the anthracyclines/anthracenediones, vosaroxin is not a substrate for P-gp [33, 48]. Vosaroxin has demonstrated activity in drug-resistant xenograft models SBC-3/ADM (doxorubicin resistance), SBD-3/ETP (etoposide resistance), and MES-SA/Dx5 (multidrug resistance) (Fig. 4) [33]. These tumor models overexpress P-gp, and SBC-3/ADM and SBD-3/ETP also have reduced expression levels of topoisomerase II. In the MES-SA/Dx5 xenograft model, vosaroxin has been shown to inhibit tumor proliferation by 87 %, compared with only 10 % inhibition by doxorubicin (Fig. 4a) [33].

Notably, the activity of vosaroxin is maintained in cells with *p53* deletion [28, 49]. Deletions and mutations in the *p53* gene are common in relapsed and treatment-related AML. Correspondingly, *p53* alterations are found frequently in AML patients with complex karyotype and in older patients; these patients often experience chemotherapy resistance and poor outcomes [50]. The ability of vosaroxin to evade two common resistance mechanisms associated with other topoisomerase inhibitors (*p53* alterations, P-gp upregulation) may contribute to the complete remissions observed in vosaroxin-treated patients with AML resistant to prior treatment with topoisomerase II inhibitors [44, 51].

Table 1 Analysis of percent growth inhibition of tumor xenograft models following exposure to vosaroxin, cisplatin, etoposide, irinotecan, doxorubicin, or paclitaxel [33]

% Inhibition	Leukemia	Lymphoma	Breast	Ovarian		Colon		Lung		Gastric			Melanoma
	CCRF-CEM	LM-3 Jck	MDA-MB-231	PA-1	SK-OV-3	WiDr	HCT116	NCI H460	Calu-6	Hs746T	GT3TKB	RF-1	SK-MEL-5
Vosaroxin													
15 mg/kg	ND	ND	80*	85*	63*	55*	63*	75*	82*	77*	69*	–13	51
20 mg/kg	ND	96*	85*	85*	71*	63*	82*	84*	88*	83*	65*	–8	55*
25 mg/kg	98*	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND
Cisplatin													
10 mg/kg	ND	3	25	33	52*	33*	13	25*	84*	55	45	40	ND
Etoposide													
12 mg/kg	28	3	45	38	14	–1	26*	31*	45*	1	37	–37	30
Irinotecan													
100 mg/kg	100*	98*	ND	94*	70*	55*	71*	64*	90*	100*	55	ND	ND
Doxorubicin													
12 mg/kg	50*	57*	44	47	20	26	40*	49*	70*	99*	46	ND	ND
Paclitaxel													
28 mg/kg	ND	ND	ND	99*	97*	97*	96*	43*	100*	100*	98*	ND	ND
42 mg/kg	100*	97*	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND	ND

ND not determined

* Statistically significant difference as evaluated by comparing the mean tumor size of the vehicle-treated groups to drug-treated groups using a two-tailed Dunnett's test. *P* values less than 0.05 were considered significant

6 Vosaroxin Clinical Development in Acute Myeloid Leukemia (AML)

The activity of vosaroxin in human leukemic cell lines and hematologic xenograft models provided the rationale for clinical study of vosaroxin in patients with hematologic malignancies. Myelosuppression was observed in preclinical toxicology studies (data on file, Sunesis Pharmaceuticals, South San Francisco, CA, USA) and was dose limiting in early clinical studies in solid tumors [52]. A phase Ib dose escalation/pharmacokinetic (PK) study was conducted in patients with advanced hematologic malignancies ($N = 73$; median age 65 years) to evaluate dosing and tolerability [53]. Grade 3 stomatitis was dose limiting for weekly (days 1, 8, 15) and twice-weekly (days 1, 4, 8, 11) regimens (28-day cycle), resulting in maximum tolerated doses of 72 and 40 mg/m^2, respectively. In this study, PK was linear over the dose range of 9–90 mg/m^2. Mean volume of distribution was 119 L, and mean half-life was approximately 25 h. Mean total body clearance was approximately 4 L/h and independent of age, sex, body weight, and body surface area. During biweekly dosing, limited drug accumulation was observed (average 1.2-fold), with no evidence of induction or inhibition of vosaroxin metabolism with repeated dosing.

In two phase II trials, vosaroxin demonstrated clinical activity in patients with AML. In the first study, three treatment schedules of single-agent vosaroxin were evaluated in an open-label, multicenter study in patients ≥60 years of age with newly diagnosed, poor-risk AML [54]. In addition, patients were required to have one or more of the following adverse prognostic factors: age ≥70 years, an antecedent hematologic disorder, Eastern Cooperative Oncology Group performance status of 2, or intermediate or unfavorable karyotype [defined as t(8;21)(q22;q22); inv(16)(p13;q22) or t(16;16)(p13;q22); or t(15;17)(q22;q12) and variants]. The primary objective of the study was to evaluate the combined complete remission (CR) rate (CR + CR with incomplete platelet recovery [CRp]) of vosaroxin in these patients. A total of 113 patients were enrolled and treated (29 patients in schedule A [72 mg/m^2 days 1, 8, and 15], 35 patients in schedule B [72 mg/m^2 days 1 and 8], and 49 patients in schedule C [29 patients at 72 mg/m^2 days 1 and 4 and 20 patients at 90 mg/m^2 days 1 and 4]). In the overall population, 36 patients (32 %) achieved CR/CRp (33 with CR and 3 with CRp), with a median overall survival (OS) of 7.0 months. Thirty-day and 60-day mortality rates were 12 and 31 %, respectively. Grade ≥3 AEs occurring in ≥20 % of patients were thrombocytopenia (59 %), febrile neutropenia (50 %), anemia (49 %), neutropenia (29 %), aggregate sepsis (39 %; aggregate of 32 preferred terms including sepsis, bacteremia, fungemia, and viremia), aggregate pneumonia (30 %; aggregate of ten preferred terms), hypokalemia (25 %),

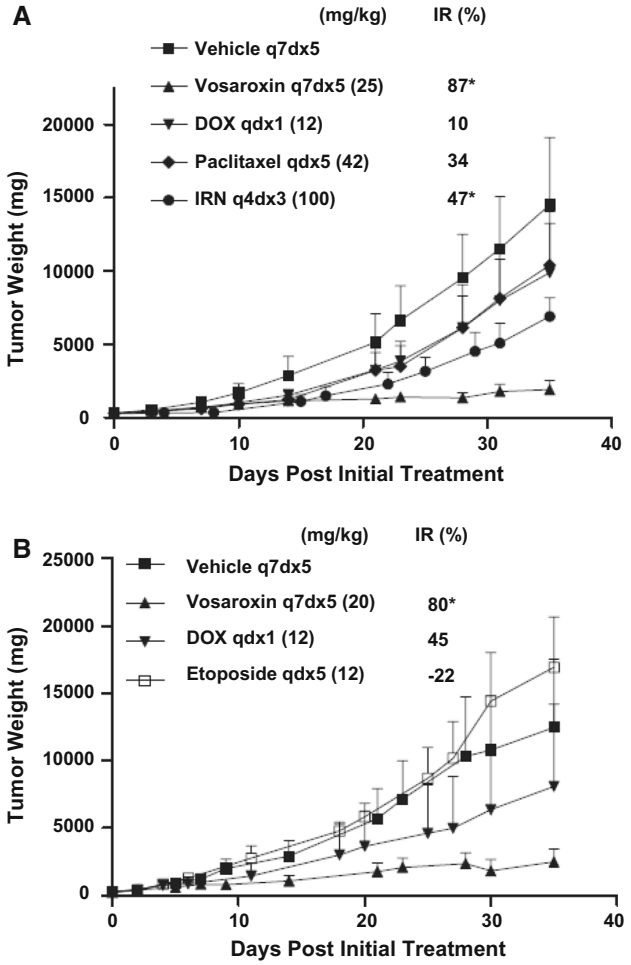

Fig. 4 Vosaroxin demonstrates potent anticancer activity in multidrug-resistant human tumor xenograft models. **a** MES-SA/Dx5. **b** SBC-3/ETP. All agents were administered intravenously using the schedules and doses indicated in the figure. Inhibition rate (IR) represents (1 − average tumor weight/average tumor weight control) × 100 as determined on day 35 after initial treatment. IR values marked with *asterisk* are statistically significantly different from those in the vehicle-treated group. Each schedule and agent had its own vehicle control group; only the vosaroxin vehicle group is shown. *Error bars* indicate one standard deviation. *CDDP* cisplatin, *DOX* doxorubicin, *IRN* irinotecan [33]

and stomatitis (22 %). Based on efficacy and safety findings, vosaroxin 72 mg/m^2 on days 1 and 4 was recommended for further study in this population. CR was achieved in 31 % of patients at this dose and schedule, with a median OS of 7.7 months, and early mortality was lowest with this dose and schedule (30- and 60-day mortality rates of 7 and 17 %, respectively).

In a second phase II trial, vosaroxin was evaluated in combination with cytarabine, based on preclinical evidence of synergistic cytotoxicity in AML cell lines and primary AML patient cells, as well as enhanced activity in a normal mouse bone marrow ablation/repopulation model [28, 45]. Lancet and colleagues evaluated the combination in this phase Ib/II, open-label, dose-escalation study in patients with R/R AML, with expansion at the maximum tolerated dose (MTD) [51]. Patients received vosaroxin (escalating doses starting at 10 mg/m^2) on days 1 and 4 in combination with cytarabine either as a 24-h continuous intravenous (CIV) infusion (400 mg/m^2/day × 5 days; schedule A) or as a 2-h IV infusion (1 g/m^2/day × 5 days; schedule B). A total of 110 patients were enrolled and 108 received any treatment. When combined with cytarabine as a 24-h CIV infusion, the MTD for vosaroxin was determined to be 80 mg/m^2 with grade 3 bowel obstruction and stomatitis as dose-limiting toxicities. When combined with cytarabine as a 2-h IV infusion, the MTD for vosaroxin was not reached; the phase II recommended dose for this combination was the highest vosaroxin dose tested, 90 mg/m^2. These doses were used in the expansion phase. Among all 108 treated patients, 24 (22 %) achieved a CR. In the efficacy population (patients with first-relapsed or primary refractory disease who received vosaroxin 80−90 mg/m^2; n =69), the CR rate was 25 % and median OS was 6.9 months (95 % confidence interval [CI] 4.3−10.1 months). Thirty-day mortality was 9.3 % (10/108) among all treated patients and 2.5 % (2/78) among patients treated at 80−90 mg/m^2; 60-day mortality was 14.8 % (16/108) and 9.0 % (7/78), respectively. Grade ≥3 non-hematologic AEs occurring in ≥15 % of all patients were aggregated sepsis/bacteremia (34 %; aggregate of 12 preferred terms), aggregated infections (19 %; aggregate of 23 preferred terms), hypokalemia (26 %), and stomatitis (15 %). PK of vosaroxin in combination with cytarabine was similar to that observed when vosaroxin was administered as a single agent, suggesting that coadministration of cytarabine did not alter vosaroxin PK. These findings supported the initiation of the pivotal phase III VALOR trial.

VALOR was a randomized, double-blind, placebo-controlled study evaluating vosaroxin (90 mg/m^2 IV, days 1 and 4; 70 mg/m^2 in subsequent cycles) plus cytarabine (1 g/m^2 IV over 2 h, days 1−5) versus placebo/cytarabine in 711 patients with R/R AML [44]. Eligible patients were considered fit to receive intensive chemotherapy and must have already tolerated induction chemotherapy with an anthracycline (or anthracenedione) plus cytarabine. Patients were stratified at randomization by age (<60 or ≥60 years), disease status (refractory, early relapse, late relapse), and region (USA, outside of USA). In this study, median OS was 7.5 months (95 % CI 6.4−8.5 months) for vosaroxin/cytarabine-treated patients and 6.1 months (95 % CI 5.2−7.1 months) for placebo/cytarabine-treated patients (hazard ratio [HR] 0.87, 95 % CI 0.73−1.02; 2-sided unstratified log-rank p = 0.061; 2-sided stratified log-rank p = 0.024). In predefined subgroup analyses, the addition of vosaroxin produced the greatest OS benefit in patients ≥60 years of age (OS 7.1 months vs. 5.0 months

with placebo/cytarabine; HR 0.75, 95 % CI 0.62−0.92; $p = 0.003$) and in those with early relapse (OS 6.7 vs. 5.2 months; HR 0.77, 95 % CI 0.59−1.00; $p = 0.039$). Overall, adding vosaroxin nearly doubled the CR rate (30 vs. 16 %; $p = 0.0001$). Similar 30-day (8 vs. 7 %) and 60-day (20 vs. 19 %) all-cause mortality rates were observed between treatment arms [44].

The safety profile of vosaroxin is consistent with non-clinical toxicology observations. In VALOR, grade ≥3 AEs were primarily related to gastrointestinal events, myelosuppression, and infection [44]. The most common grade ≥3 events (experienced by ≥10 % of patients treated with vosaroxin/cytarabine) were febrile neutropenia (47 % with vosaroxin/cytarabine vs. 34 % with placebo/cytarabine), thrombocytopenia (24 vs. 25 %), anemia (22 vs. 23 %), neutropenia (19 vs. 14 %), hypokalemia (15 vs. 6 %), pneumonia (11 vs. 8 %), stomatitis (16 vs. 3 %), sepsis (12 vs. 5 %), and bacteremia (12 vs. 5 %). Serious AEs were more frequent in the vosaroxin arm: febrile neutropenia (11.3 vs. 7.4 % with placebo/cytarabine), sepsis (8.7 vs. 4.3 %), pneumonia (7.6 vs. 4.9 %), bacteremia (8.5 vs. 2.9 %), and stomatitis (3.4 vs. 1.4 %) [55]. Overall, the primary toxicities of vosaroxin are similar to those observed with many antineoplastic cytotoxic drugs. However, cardiac, pulmonary, renal, and hepatic AEs were comparable between arms, and no clinical evidence was seen with vosaroxin for such off-target end-organ toxicities, suggesting a possible association with the stability of vosaroxin's quinolone core and minimal production of toxic metabolites.

In the clinical setting, quinolone antibiotics have been associated with nausea, diarrhea, headache, and dizziness. Rarely, severe AEs such as QTc interval prolongation, tendonitis/tendon rupture, disturbances in glucose homeostasis, crystalluria, interstitial nephritis, acute renal failure, seizures, and class-specific phototoxicity have been reported [56]. The incidence of these toxicities in the VALOR trial was evaluated because vosaroxin is a quinolone derivative; in general, similar frequencies were observed between treatment arms.

Additionally, interim data from a phase II, multicenter, randomized, open-label study (LI-1) using the "Pick a Winner" design were reported for newly diagnosed patients aged ≥60 years for whom intensive therapy was not suitable [57]. Two vosaroxin-based regimens were compared with low-dose cytarabine (LDAC): (a) single-agent vosaroxin (72 mg/m^2 IV days 1 and 4, up to four cycles) versus LDAC (20 mg subcutaneously twice daily, days 1−10 for at least four cycles); and (b) vosaroxin plus LDAC versus LDAC. Selection of the vosaroxin dose (72 mg/m^2 IV days 1 and 4, up to four cycles) was based on the efficacy and safety results for single-agent vosaroxin in newly diagnosed older patients in the phase II study by Stuart and colleagues [54]; synergy observed in preclinical studies provided the rationale for the combination with LDAC [57]. A total of 104 randomized patients were included in each comparison. Mean patient age across all arms was 75 years (range 60−91 years). Advanced age was the primary reason patients were considered not to be candidates for intensive therapy (and thus were eligible for enrollment), followed by advanced age with poor performance status. At the first interim analysis, single-agent vosaroxin did not meet the prespecified hurdle of 2.5 % absolute improvement in CR rate over LDAC alone (15 % with vosaroxin vs. 16 % with LDAC), leading to closure of this study cohort. On the other hand, vosaroxin in combination with LDAC met the prespecified 2.5 % improvement in CR rate at the first interim analysis (25 % with vosaroxin/LDAC vs. 20 % with LDAC). In spite of this improvement, the data monitoring and ethics committee recommended closure of this cohort based on preliminary OS and early mortality data available at the time of the interim analysis. In the randomization between single-agent vosaroxin versus LDAC, 30- and 60-day mortality rates were higher with single-agent vosaroxin than with LDAC (30-day: 26 vs. 14 %, respectively; 60-day: 38 vs. 20 %) and OS was shorter in the vosaroxin arm (HR 1.94 [95 % CI 1.26−3.00]). In the vosaroxin/LDAC versus LDAC randomization, 30-day mortality rates were similar between arms (10 % with vosaroxin/LDAC vs. 11 % with LDAC) while 60-day rates were higher with combination therapy (36 vs. 18 % with LDAC); OS was not significantly different between arms at the interim analysis (HR 1.30 [95 % CI 0.81−2.07]). The investigators concluded that treatment with vosaroxin was "more intensive than anticipated" and was unlikely to benefit older AML patients not considered candidates for intensive therapy. The differences between findings in the LI-1 study and VALOR are likely related to differences between the two study populations. The LI-1 study comprised high-risk (advanced age, poor performance status) newly diagnosed AML patients who were not considered fit for intensive therapy, whereas the VALOR study comprised R/R AML patients who had all previously received intensive chemotherapy and were selected to received additional intensive therapy in the R/R setting.

Additional trials are ongoing to determine the setting and combination that best translates vosaroxin activity into a clear survival benefit. Promising results have been presented from an ongoing phase I/II open-label, single-arm, investigator-sponsored trial evaluating the safety and clinical activity of vosaroxin in combination with decitabine in patients ≥60 years of age with previously untreated AML or high-risk myelodysplastic syndromes (MDS) [58]. This trial consists of a lead-in phase I portion to determine a safe dose of vosaroxin (using a starting dose of vosaroxin 90 mg/m^2 IV on days 1 and 4) in combination with decitabine 20 mg/m^2 IV on days 1−5, followed by a phase II expansion. A total of 62 patients (55 with AML, seven with

high-risk MDS) have been enrolled with a median patient age of 69 years (range 60−78). Vosaroxin at the 90 mg/m^2 dose level was well tolerated in the first six patients enrolled in the phase I portion of the trial; however, eight episodes of grade 3/4 mucositis occurred among the next 16 patients enrolled, and the vosaroxin dose was subsequently reduced to 70 mg/m^2. Among all 62 enrolled patients, the overall response rate (ORR) was 74 %, including CR in 31 patients (50 %), with a median OS of 9.8 months. The reduction of the vosaroxin dose to 70 mg/m^2 ($n = 40$) from 90 mg/m^2 ($n = 22$) was associated with reduced 8-week mortality (8 % with 70 mg/m^2 vs. 23 % with 90 mg/m^2), similar ORR (75 vs. 73 %, respectively), and improved OS (median OS of 11.5 vs. 5.5 months, respectively). Therapy-related grade $\geq$3 toxicities included mucositis in 11 patients (18 %) and liver enzyme elevation in eight patients (13 %).

7 Conclusions and Clinical Impact

Vosaroxin is the first of a new class of anticancer agents and is the first quinolone-based topoisomerase II inhibitor studied in clinical trials in cancer. Vosaroxin is a DNA intercalating topoisomerase II inhibitor that causes the induction of apoptosis via double-strand DNA breaks; it is chemically distinct from other topoisomerase inhibitors with a stable quinolone-based core. Unlike etoposide, mitoxantrone, and the anthracyclines, vosaroxin's activity appears to be exclusively attributable to intercalation and topoisomerase II inhibition, lacking cytotoxicity due to metabolic activation and oxidative stress. The lack of significant toxic metabolites, free radicals, and ROS may be the basis for the low incidence of cardiac, pulmonary, renal, and hepatic toxicities. Furthermore, vosaroxin demonstrates potent in vitro antitumor activity in various tumor types including those resistant to other topoisomerase II inhibitors.

In the pivotal phase III VALOR trial, a 2.1-month improvement in OS among patients $\geq$60 years old was demonstrated, with low early mortality. Common side effects of vosaroxin included gastrointestinal effects, myelosuppression, and infection. Vosaroxin may be an effective therapeutic alternative for older AML patients, those with treatment-resistant disease, and those who have exceeded safe thresholds for anthracyclines or are at high risk for treatment-related cardiac damage. Overall, vosaroxin represents a much needed novel treatment for patients with R/R AML.

Compliance with Ethical Standards

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Conflict of interest GCJ is employed by Sunesis Pharmaceuticals. JAF was previously employed by and is a consultant for Sunesis Pharmaceuticals. MP has no conflicts of interest to disclose. SAS has served on advisory boards and a Qinprezo (vosaroxin) Steering Committee with Sunesis Pharmaceuticals. In addition, he has received research funding to support efforts related to an ongoing investigator-initiated trial involving the use of vosaroxin in previously untreated AML patients.

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