The treatment of acute myeloid leukemia (AML) in adults has not significantly changed in the past five decades. Despite the availability of effective induction chemotherapy, most patients eventually relapse. Relapsed or refractory AML, and AML in patients 60 years of age and older are particularly difficult to treat and have poor prognosis hence the need for novel therapies. Vosaroxin is a novel first-in-class anticancer quinolone derivative that intercalates DNA and inhibits topoisomerase II. Vosaroxin was shown in preclinical studies to be a potent antileukemia agent with unique features that overcome common mechanisms of resistance to standard therapy. Vosaroxin as single agent or in combination with cytarabine showed promising tolerability and activity in Phase I and II studies. The combination of vosaroxin and cytarabine is currently being studied in the Phase III VALOR trial that will define the role of vosaroxin in first relapsed or refractory AML. This review summarizes the preclinical and clinical studies of vosaroxin in AML.

Keywords: acute myeloid leukemia • quinolones • voreloxin • vosaroxin

Acute myeloid leukemia (AML) is the most common acute leukemia diagnosed in adult patients [1]. Although the majority of patients achieve remission with induction chemotherapy, most of them will eventually relapse. The uses of consolidation chemotherapy and stem cell transplantation improve outcome in adult AML but the prognosis of the disease remains poor, especially in the older population and in patients with treatment-related AML [2–4]. Among patients ≥60 years of age, 40–50% of those in good performance status achieve complete remission but the cure rate is <10% and the median survival is less than 1 year [5]. The treatment for newly diagnosed AML has not changed significantly over the past few decades [6]. The combination of an anthracycline with cytarabine remains the mainstay of treatment. Vosaroxin is one of the emergent novel therapies in AML that was shown in preclinical and Phase I and II clinical trials to have antileukemic properties and is currently being studied in the Phase III setting.

Mechanism of action
Quinolones are a class of drugs commonly used clinically as antibacterial agents rather than antineoplastic agents. Quinolones induce DNA damage in bacteria by interfering with DNA gyrase and topoisomerase IV, which are the functional analogues of topoisomerase II in eukaryotes [7–9].

Topoisomerase II is essential for the survival of eukaryotic cells [10,11]. It functions as a homodimer, generating double-stranded breaks and regulating the super-coiling of the DNA. It acts by passing an intact DNA double helix through another double helix that has been cleaved by the enzyme requiring a complex conformational change. Topoisomerase II religates the cleaved strand following DNA strand passage [12,13]. The expression of this enzyme peaks at the G2/M phase of the cell cycle [14]. Topoisomerase II is a target of many antineoplastic
drugs, including the anthracyclines and etoposide [15–17]. There are two classes of topoisomerase II inhibitors in current use clinically. One class that includes the anthracyclines and anthracenediones, intercalate DNA and inhibit topoisomerase II; the other class is represented by the epipodophyllotoxins etoposide and teniposide, that do not intercalate DNA but inhibit enzymatic function directly [15,16]. Tomita et al. screened several antibacterial agents with a quinolone-like ring structure for possible antineoplastic activity and later optimized a class of compounds bearing a 1,8-naphthyridine core for cytotoxicity [18,19]. Vosaroxin had the best anticancer activity of these compounds.

Vosaroxin (previously known as voreloxin, SNS-595, AG-7532) is a novel first-in-class anticancer quinolone derivative. It is a topoisomerase II inhibitor that acts by intercalating DNA and inhibiting topoisomerase II, leading to DNA double strand breaks, G2 arrest and apoptosis [14]. The vosaroxin-induced DNA damage is, in contrast to anthracyclines, site selective targeting GC-rich regions (Figure 1). Another differentiating feature is that vosaroxin is less chemically reactive compared with anthracyclines, generating fewer free radical metabolites and reactive oxygen species and may have lower potential for cardiotoxicity [14]. The efficacy of anthracyclines and mitoxantrone are limited by their sensitivity to the common tumor resistance mechanism of P-glycoprotein (P-gp) efflux [20]. The expression of P-gp is an independent poor prognostic factor for response to therapy in AML, and higher levels are expressed in both older and relapsed patients [21,22]. In contrast to anthracyclines and mitoxantrone, vosaroxin is not a P-gp substrate [23] and has shown activity in preclinical models of drug resistance and in patients refractory to prior therapy with anthracyclines and other topoisomerase II inhibitors.

In preclinical studies, vosaroxin demonstrated potent activity in a variety of mouse models, including

Figure 1. Vosaroxin: mechanism of action and structural formula. (A) The mechanism of action of vosaroxin. Vosaroxin acts by intercalating DNA at GC-rich regions, inhibiting topoisomerase-2 and leading to cell cycle arrest at G2/M leading to apoptosis. (B) The structural formula of vosaroxin.
drug-resistant tumors, normal mouse models of marrow ablation, and syngeneic and human xenografts of various tissue origins [23,24]. The activity of vosaroxin was initially established in solid tumors. Hoch et al. tested vosaroxin against 11 human cell lines of breast, bladder, pancreatic, nasopharyngeal, colon, ovary, stomach, lung and uterine origin and its activity was compared with a variety of chemotherapeutic agents including etoposide, doxorubicin, irinotecan, cisplatin, paclitaxel and 5-fluorouracil. The activity of vosaroxin in these models was superior to conventional chemotherapy and it was found to be among the most potent agents tested [23]. With multiple potential targets for solid tumors identified, Scatena et al. examined its activity against hematologic cancer cell lines [24]. Cell viability assays using human cell lines derived from acute promyelocytic leukemia (HL-60), acute lymphoblastic leukemia (CCRF-CEM) and biphenotypic leukemia (MV4–11) demonstrated that both vosaroxin and cytarabine have potent activity against all these cell lines. When cells were treated with both cytarabine and vosaroxin, a synergistic effect was seen in myeloid and promyelocytic leukemia cell lines and an additive effect was seen in the lymphoblastic leukemia cell line. In a normal mouse model of bone marrow ablation and repopulation that mimics the AML treatment paradigm, vosaroxin was more effective than cytarabine in decreasing bone marrow cellularity at maximum tolerated doses of these agents. Greater than additive activity was seen when vosaroxin and cytarabine were combined in this model. Complete bone marrow repopulation and peripheral blood count recovery was observed [24]. Walsby et al. examined the cytotoxic effects of vosaroxin on mononuclear cells from patients with newly diagnosed AML. Vosaroxin was found to have an IC50 less than half of that of cytarabine indicating it as a potent inhibitor of myeloid cell growth, and synergy between vosaroxin and cytarabine was observed [25]. These data supported the clinical investigation of vosaroxin in combination with cytarabine. The activity of vosaroxin is independent of p53, and given that it is not a P-gp substrate, vosaroxin may bypass common mechanisms of resistance to chemotherapy [26,27]. Mutation of Flt-3 is well characterized in AML and is commonly due to internal tandem duplication (ITD) of exons 14 and 15, leading to the constitutive activation of the enzyme, carrying a poor prognosis and creating a potential target for the treatment of Flt-3-mutated AML [28]. Vosaroxin was shown to be more effective than cytarabine at inhibiting the growth of the MV4–11 cell line harboring the Flt3-ITD mutation suggesting that vosaroxin could play a potential therapeutic role against the Flt3-ITD positive AML [24].

Clinical studies

■ Phase IB study

A single-agent Phase IB dose-escalating study of vosaroxin evaluated its dose-limiting toxicity, maximum-tolerated dose (MTD), pharmacokinetics, clinical activity and pharmacodynamics in relapsed/refractory leukemias [29]. Two dosing schedules were evaluated. The first one was weekly (days 1, 8 and 15) and the second one was twice weekly (days 1, 4, 8 and 11). A total of seventy-three patients were enrolled in the trial. The median age was 65 years and 85% of the patients had AML of whom 78% had refractory disease. In the weekly schedule the MTD was 72 mg/m²; in the twice-weekly schedule the MTD was 40 mg/m². The dose-limiting toxicity was stomatitis. Primary nonhematologic toxicities were infections, febrile neutropenia and stomatitis. The 30-day all-cause mortality was 11%. Clearance of blasts from the bone marrow was seen in 22% of the treated patients and a third of these went into complete remission (CR) or CR without platelet recovery. The median duration of remission was 3.1 months.

■ Phase IB/II study

The combination of vosaroxin with cytarabine was studied in a Phase IB/II study in relapsed/refractory AML patients (n = 108) [30]. In the Phase IB dose escalation part of the study, vosaroxin was given on days 1 and 4 by intravenous infusion within 10 min, in combination with two schedules of cytarabine. The first cytarabine dose schedule was a continuous infusion of cytarabine at 400 mg/m² daily for 5 days in combination with vosaroxin over a dose range of 10–90 mg/m². The second dose schedule was a 2 h intravenous infusion of cytarabine at 1 g/m²/day given daily for 5 days and the vosaroxin dose range was 70–90 mg/m². The MTD for vosaroxin with the continuous infusion cytarabine regimen was 80 mg/m², and the recommended Phase II dose for vosaroxin in combination with the second iv. cytarabine infusion was 90 mg/m². In the Phase II expansion study of first relapsed and primary refractory AML populations, an overall remission rate (ORR; CR plus CR without platelet recovery) of 28% was seen, with 25% CR. An ORR of 33 and 21% was seen in patients with first relapsed AML and in patients with AML refractory to initial therapy respectively; 26% of patients received HSCT following vosaroxin/cytarabine treatment. Thirty- and sixty-day all-cause mortality was 3 and 9%, respectively, and the median overall survival was 6.9 months [31].

■ Phase II study

The REVEAL-1 study

The Phase II REVEAL-1 trial is a multicenter trial that studied single-agent vosaroxin in three dosing
schedules in elderly patients with newly diagnosed and previously untreated AML [32,33]. The trial included a total of 113 patients 60 years of age or older with at least one additional adverse risk factor such as age greater than 70 years, intermediate or unfavorable cytogenetics, secondary AML, or Eastern Cooperative Oncology Group performance status greater or equal to two. The median age of the enrolled patients was 74 years and more than 80% of the patients had two or more adverse risk factors. Four sequential dose regimens were studied: vosaroxin administered at 72 mg/m² weekly for 3 weeks, (n = 29), 72 mg/m² weekly for 2 weeks (n = 35), or 72 mg/m² on days 1 and 4 (n = 29) or 90 mg/m² vosaroxin on days 1 and 4 (n = 20). Common nonhematologic toxicities included febrile neutropenia (50%), pneumonia (30%) and bacteremia/sepsis (39%). ORR across all schedules was 32%, with 78% of patients who achieved remission having done so during the first cycle. For patients who received reinduction, CR was seen in 29%. Median overall survival was 7.0 months. Although response rates were highest for the weekly (41%) and the days 1 and 4 schedules at 72 mg/m² vosaroxin (35%), the safety profile appeared superior in the latter schedule. This was reflected in lower 30-day all-cause mortality (6.9%) for the days 1 and 4 at 72 mg/m² compared with that of the weekly schedule (21%). The days 1 and 4 schedule at 72 mg/m² was recommended for further clinical investigation.

Table 1 summarizes the key findings of the completed clinical trials of vosaroxin in AML.

### Phase III studies
The VALOR study
The Phase III VALOR trial is a randomized, controlled, double-blinded multinational study examining cytarabine plus vosaroxin or placebo for relapsed or refractory AML. Enrollment began in December 2010 and was completed in September 2013 [101]. Eligible patients should have either refractory AML or first relapsed AML. The VALOR study was a double-blinded, randomized, controlled, multinational trial examining cytarabine plus vosaroxin or placebo for relapsed or refractory AML. Enrollment began in December 2010 and was completed in September 2013 [101]. Eligible patients should have either refractory AML or first relapsed AML.

<table>
<thead>
<tr>
<th>Phase</th>
<th>Number of patients</th>
<th>Arms</th>
<th>Patient population</th>
<th>Key findings</th>
<th>Adverse events</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phase IB 73</td>
<td>Weekly vosaroxin versus twice weekly vosaroxin</td>
<td>Advanced hematologic malignancies; 78% refractory AML</td>
<td>CR + CRp: 7% clearance of blasts: 22%, Median duration of remission: 3.1 months</td>
<td>Infections, febrile neutropenia, stomatitis (oral mucositis)</td>
<td>[29]</td>
<td></td>
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<tr>
<td>Phase IB 57</td>
<td>Vosaroxin days 1, 4 + cytarabine 400 mg/m² continuous intravenous on days 1–5 and vosaroxin days 1, 4 + cytarabine 1 g/m² over 2 h days 1–5</td>
<td>Relapsed/refractory AML</td>
<td>ORR 25%. Vosaroxin dose established at 80 mg/m² if cytarabine given as continuous infusion and 90 mg/m² if cytarabine given as 2-h intravenous infusions</td>
<td>Sepsis/bacteremia, infections, stomatitis (oral mucositis)</td>
<td>[30]</td>
<td></td>
</tr>
<tr>
<td>Phase II 69†</td>
<td>Vosaroxin 80 mg/m² days 1, 4 + cytarabine 400 mg/m² continuous intravenous days 1–5 vs vosaroxin 90 mg/m² days 1, 4 + cytarabine days 1 g/m² over 2 h days 1–5</td>
<td>Relapsed/refractory AML</td>
<td>ORR 28%, median OS 6.9 months</td>
<td>Sepsis/bacteremia, infections, stomatitis (oral mucositis)</td>
<td>[31]</td>
<td></td>
</tr>
<tr>
<td>Phase II (REVEAL) 113</td>
<td>Vosaroxin 72 mg/m² weekly for 3 weeks, 72 mg/m² weekly for 2 weeks, 72 mg/m² on days 1 and 4, vosaroxin at 90 mg/m² on days 1 and 4</td>
<td>Newly diagnosed, previously untreated AML, age ≥60 years + one additional adverse risk factor</td>
<td>ORR 32% and 72 mg/m² days 1, 4 ORR 35%, 30-day all-cause mortality 6.9%, OS 7.7 months</td>
<td>Febrile neutropenia, pneumonia, bacteremia/sepsis, stomatitis (oral mucositis)</td>
<td>[32,33]</td>
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</tr>
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</table>

†Included first relapsed and refractory patients from dose escalation who received 80 or 90 mg/m² vosaroxin.

AML: Acute myeloid leukemia; CR: Complete remission; CRp: Complete remission with incomplete platelet recovery; ORR: Overall response rate; OS: Overall survival.
AML (status/post 1–2 induction chemotherapy cycles including one cycle of cytarabine in combination with either an anthracycline or anthracyclenedione). The treatment in this trial consisted of intermediate dose cytarabine given at 1000 mg/m² daily by a 2 h iv. infusion on days 1–5 with either vosaroxin at 90 mg/m² or placebo by a 10 min iv. infusion on days 1 and 4. Randomization was carried on a 1:1 basis. After the first induction cycle, the vosaroxin dose is attenuated to 70 mg/m² for subsequent cycles (up to four cycles allowed: a second induction cycle and up to 2 cycles of consolidation). The primary end point is overall survival and the secondary end point is CR rate. Additional endpoints include stem cell transplantation rates and safety. The initial sample size was 450 [34]. At the interim analysis in September 2012, the Data and Safety Monitoring Board recommended implementing the preplanned adaptive design, increasing target sample size to 675 patients. The results of this study are eagerly awaited.

The LI-1 trial
The Leukemia Lymphoma Research and NCRI Working Group Pick a Winner Program (LI-1) is a multicenter Phase II/III study that is currently enrolling patients (ISRCTN40571019). Eligible patients are those with AML or high-risk myelodysplastic syndrome (RAEB-2) deemed unfit for standard induction chemotherapy. The trial is evaluating five novel treatment arms in comparison to low-dose cytarabine. One arm evaluated vosaroxin as a single agent and a second arm is investigating vosaroxin in combination with low-dose cytarabine. The vosaroxin dose regimen is 72 mg/m² by iv infusion within 10 min on days 1 and 4 for both treatment arms. Patients may receive four or more cycles of treatment. End points of the trial are overall survival, CR rates and safety in addition to quality of life. The vosaroxin single-agent arm was completed and the data monitoring committee recommended discontinuing further enrollment; the vosaroxin combination with low-dose cytarabine is ongoing.

Safety & tolerability
As outlined in the previous sections the dose-limiting toxicity of vosaroxin is stomatitis. Nonhematologic toxicities were mainly febrile neutropenia, infections and gastrointestinal symptoms. Importantly, vosaroxin caused no significant neurologic, hepatic, renal or cardiac toxicity, which makes its combination with other chemotherapeutic agents an appealing option.

Future perspective
The outcome of patients with AML remains dismal especially in the elderly population and the development of new therapies are needed. Vosaroxin has been evaluated both as single agent and in combination with cytarabine. Several unique features of vosaroxin make it a potentially attractive chemotherapy agent against difficult to treat refractory and relapsed AML. These properties include its resistance to the P-gp-mediated drug efflux, its lack of production of reactive oxygen species species, its activity against p53-mutant and Flt3-ITD-mutant AML. These properties offer advantages as compared with the conventional anthracyclines. In addition, its ease of administration and its ability to be combined safely and synergistically with cytarabine make it an appealing agent in the treatment of AML. The results of the ongoing VALOR trial in adult patients of all ages will be critical for the future use of vosaroxin in AML as it will help define the category of patients who might benefit from the drug and its adaptive design will aid in the detection of a possible survival benefit.

Executive summary
Mechanism of action
- Vosaroxin is a novel first-in-class anticancer quinolone derivative that acts as a topoisomerase II inhibitor and acts by intercalating DNA at GC-rich regions causing cell cycle arrest and apoptosis.

Clinical trials
- Phase I/II trials showed a benefit of vosaroxin in refractory acute myeloid leukemia both as single agent and in combination with cytarabine.
- Vosaroxin has advantages over conventional chemotherapy including its resistance to the P-gp-mediated drug efflux, its lack of production of free radical and reactive oxygen species species, its activity against p53 mutant and Flt3-ITD mutant acute myeloid leukemia.

Future perspective
- The results of the ongoing Phase III trial (VALOR) will determine its future role in relapsed and refractory acute myeloid leukemia.
References

Papers of special note have been highlighted as: ■ of interest


Important paper that initially demonstrated preclinical activity of vosaroxin in cancer cell lines and in vivo tumor models.


Important paper that first demonstrated preclinical activity of vosaroxin in hematopoietic cell lines.


Important Phase II study (the REVEAL study) that established the role of single agent vosaroxin in acute myeloid leukemia.
33. Stuart RK, Kashani FR, Cripe ID et al. Voreloxin single-agent treatment for older patients (60 years or older) with previously untreated acute myeloid leukemia: Final results from a Phase II study with three schedules. Presented at: 2010 ASCO Annual Meeting. IL, USA, 4–8 June 2010.

Website