PROTOCOL INFORMATION

Study Title: Phase II trial of vosaroxin in combination with infusional cytarabine in patients with untreated AML

(VITAL Trial … Vosaroxin and Infusional Cytarabine for Frontline Treatment of Acute Myeloid Leukemia)

Sponsoring Institution: Vanderbilt-Ingram Cancer Center
Vanderbilt University Medical Center

Study Chairs:

Stephen A. Strickland, MD, MSCI
stephen.strickland@vanderbilt.edu

Michael R. Savona, MD, FACP
michael.savona@vanderbilt.edu

Statisticians: Gregory D. Ayers, MS
Jeffrey D. Blume, PhD

VICC Multi-Institutional Coordinating Center:

Address: 2141 Blakemore Ave., Nashville, TN 37212
Email address: Coordinating.Center@vanderbilt.edu
Fax no: 615-875-0040
Phone no: 615-936-5770

Contracting Authority:

Name: Amy Griffith
Address: 2141 Blakemore Avenue
Phone no: 615-875-0044
Fax no: 615-936-5850
Email Address: amy.griffith@vanderbilt.edu

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PROTOCOL ACCEPTANCE PAGE

Protocol Title:	Phase II trial of vosaroxin in combination with infusional cytarabine in patients with untreated AML
Protocol Date:	18 January 2016
Version Number:	1.10

I have received and read the above protocol. I agree to conduct the study in accordance with the current protocol.

_____ _____

Principal Investigator's Signature Date

_____

Principal Investigator's Name (Print)

Site Name _____

List of Abbreviations

AE	Adverse event
ANC	Absolute neutrophil count
AHD	Antecedent hematological disorders
ALT	Alanine aminotransferase
AML	Acute myeloid/ myelogenous leukemia
AML-MLD	Acute myeloid/ myelogenous leukemia with multilineage dysplasia
APL	Acute promyelocytic leukemia
ara-C	Cytosine arabinoside
ara-CTP	Arabinosyl-cytosine triphosphate
AST	Aspartate aminotransferase
AQD	Anticancer quinolone derivative
ASCO	American Society of Clinical Oncology
BSA	Body surface area
C	Celsius
CBC	Complete blood count
CNS	Central nervous system
CI	Confidence interval
CR	Complete remission
CRi	Complete remission with incomplete blood count recovery
CRp	Complete remission with incomplete platelet recovery
CTCAE	Common Terminology Criteria for Adverse Events
CVA	Cerebral Vascular Accident
DFS	Disease- free survival
D5W	Dextrose in water
dL	Deciliter
DLT	Dose-limiting toxicity
DSB	Double-strand breaks
DSMB	Data Safety Monitoring Board
DSMC	Data and Safety Monitoring Committee
Echo	Echocardiogram
ECOG	Eastern Cooperative Oncology Group
EFS	Event-free survival
EKG	Electrocardiogram
EOT	End of treatment
F	Fahrenheit
FDA	Food and Drug Administration
FSH	follicle-stimulating hormone
FISH	Fluorescence in situ hybridization
GCP	Good clinical practice
GVHD	Graft versus host disease
Hgb,	Hemoglobin
HP	Heavily pretreated
H & P	Medical history & physical examination
HR	Hazard ratio
HSCT	Hematopoietic stem cell transplantation
ICF	Informed consent form
IEC	Institutional Ethics Committee
IRB	Institutional Review Board
IUD	Intrauterine device

List of Abbreviations

IUS	Intrauterine system
IV	Intravenous
IWG	International Working Group
LDH	Lactate dehydrogenase
LFS	Leukemia-free survival
LVEF	Left ventricular ejection fraction
LR	Log-likelihood ratio
MDS	Myelodysplasia
mg	Milligram
MI	Myocardial infarction
MP	Minimally pretreated
MPN	Myeloproliferative neoplasms
MRD	Minimal residual disease
MTD	Maximum-tolerated dose
MUGA	Multigated acquisition
NCCN	National Comprehensive Cancer Network
NCI	National Cancer Institute
OS	Overall survival
PE	Physical examination
PET	Positron emission tomography
Plts	Platelets
PK	Pharmacokinetics
PO	"by mouth"
PR	Partial remission
PS	Performance status
PT/PTT INR	Prothrombin time/partial thromboplastin time international normalized ratio
q	Every
RBC	Red blood cell count
SAE	Serious adverse event
SRC	Scientific Review Committee
VITAL Trial	**V**osaroxin and **I**nfusional Cytarabine for Frontline **T**reatment of **A**cute Myeloid **L**eukemiaTrial
7+V	Vosaroxin and standard dose infusional cytosine arabinoside (ara-C)
t-AML/t-MDS	Treatment-related myeloid neoplasms
TF	Treatment Failure
TIA	Transient ischemic attack
TK	Tyrosine kinase
VICC	Vanderbilt-Ingram Cancer Center
WBC	White blood cell count
WHO	World Health Organization

PROTOCOL SYNOPSIS

TITLE: Phase II trial of vosaroxin in combination with infusional cytarabine in patients with untreated AML

OBJECTIVES:

Primary Objective:

- To assess the rate of complete remission (CR) after induction therapy with the combination of "7+V" [vosaroxin and standard dose infusional cytosine arabinoside (ara-C)] for patients with newly diagnosed, previously untreated acute myelogenous leukemia (AML)

Secondary Objectives:

- To further evaluate the safety and tolerability of "7+V"
- To evaluate for the presence of minimal residual disease (MRD) remaining after "7+V" induction and/or re-induction
- To determine the CR/CRi rate after one or 2 cycles of "7+V" induction
- To determine the time to neutrophil and platelet recovery following "7+V" induction
- To assess disease-free survival (DFS) at 1yr of patients achieving CR/CRi after "7+V" induction
- To assess overall survival (OS) at 1yr of all patients receiving protocol-defined therapy
- To determine the correlation of HSCT comorbidity index, AML-Score, and Wheatley Index scores with disease response, DFS and OS

Exploratory Objectives:

- To describe the mutational burden of this cohort of AML patients
- To correlate genomic aberration with response rate, DFS, and OS
- To determine the number of patients treated with vosaroxin who eventually go to allogeneic HSCT

STUDY DESIGN:

- Single-arm, open-label, phase II study of vosaroxin in combination with cytarabine in patients with newly diagnosed AML.
- Vosaroxin will be administered intravenously at 90 mg/m2 on days 1 and 4. Cytarabine will be administered in standard fashion as a continuous infusion of 100 mg/m2 daily on days 1-7.
  - Patients with evidence of residual leukemia on "Day 14 biopsy" following initial induction will be offered re-induction with intravenous vosaroxin at 70 mg/m2 on days 1 and 4 in combination with continuous infusion cytarabine at 100 mg/m2 daily on days 1-7.
- Two-stage design. Forty-one (41) patients will provide 80% power to detect a 20% increase in complete remission rates (from 40% to 60%) with a Type I Error rate of 5%. The design permits one interim look to examine evidence of futility after the first 17 patients are evaluable for response. If ≤ 7 patients achieve CR then the likelihood of reaching the goal endpoint of ≥ 60% remission would be diminished. In this case, the VITAL Study DSMB (consisting of a representative from the funding organization, the study chairs, a biostatistician, and participating investigator to be selected by the study chairs) will review the clinical data to determine the merits of continued enrollment. If > 7 CRs are observed then the second stage will open automatically and increase enrollment to 41 patients.
  - Stage 1: First 17 patients who are evaluable for response prior to futility analysis
  - Stage 2: Patients 18-41 enrolled after futility analysis
- Likelihood-based assessment of the statistical evidence be used to monitor the false discovery rate as data accrue and will provide the option of adding one or two extension cohorts of 10 participants each if the initial trial results are inconclusive.
- Selected Standard of Care Study Procedures:
  - Pre-enrollment marrow aspirate / biopsy to confirm AML diagnosis in accordance with WHO define diagnostic criteria, and to be used for analyses as outlined below in correlative analyses section
  - Echo / MUGA to assess LVEF prior to exposure to induction chemotherapy
  - Repeat marrow aspirate / biopsy at day 14 (± 2 days) following initiation of induction therapy to assess disease status
  - Recovery marrow assessment to be performed following count recovery (ANC > 1000, Platelets > 100) following the initiation of induction therapy for formal response assessment. Disease assessment will be performed on day 57 (± 3 days) for those failing to demonstrate peripheral count recovery, or per the discretion of the investigator.

STUDY POPULATION:

- Adult patients with newly diagnosed, previously untreated, acute myeloid leukemia

INCLUSION CRITERIA:

- Age ≥ 18 years of age
- Ability to provide informed consent
- Ability to tolerate intensive therapy with vosaroxin 90mg/m2 and standard dose of cytarabine as per the investigator discretion
- ECOG performance status 0-2 at time of study entry (Appendix A)
- Morphologically confirmed new diagnosis of AML in accordance with WHO diagnostic criteria (Appendix B)
- Patients who have received hydroxyurea alone or have previously received "non-cytotoxic" therapies for MDS or MPN (e.g., thalidomide or lenalidomide, 5-azacytidine or decitabine, histone deacetylase inhibitors, low-dose cytoxan, tyrosine kinase or dual TK/src inhibitors) will be allowed
- Renal function: Serum creatinine ≤ 2.0 mg/dL
- Hepatic enzymes (ALT, AST) ≤ 2.5 x upper limit of normal
- Total bilirubin ≤ 1.5 x upper limit of normal unless clearly related to Gilbert's Disease, hemolysis or leukemic infiltrate

- For patients in **stage 1 (patients #1-#17)**.
  - ≥ 55 years of age with AML of any risk classification, OR
  - 18-54 years of age with high-risk AML disease based on one of the following:
    - Antecedent hematologic disorder including myelodysplasia (MDS)-related AML (MDS/AML) and prior myeloproliferative disorder (MPD)
    - Treatment-related myeloid neoplasms (t-AML/t-MDS)
    - AML with FLT3-ITD
    - Myeloid sarcoma
    - AML with multilineage dysplasia (AML-MLD)
    - Adverse cytogenetics (defined as -5/-5q; -7/-7q; abnormal 3q, 9q, 11q, 20q, 21q or 17p; t(6;9); t(9;22); trisomy 8; trisomy 13; trisomy 21; complex karyotypes (≥ 3 clonal abnormalities); monosomal karyotypes

- For patients in **stage 2 (enrolled patient #18 and beyond)**.
  - ≥ 55 years of age with AML of any risk classification, OR
  - 18-54 years of age with intermediate or high risk AML as defined by NCCN risk assignment (Appendix C)

EXCLUSION CRITERIA:

Stage I:

- Patients 18-54 years of age with "good risk" AML defined as the presence of t(8;21) , inv(16), or t(16;16) as diagnosed by morphologic criteria, flow cytometric characteristics, and rapid cytogenetics or FISH *(Patients with t(8;21), inv(16), t(16;16) who are unable to receive anthracycline based induction will be allowed to enroll provided the medical reason they are unable to receive anthracyclines is clearly documented and provided they fulfill all other eligibility and criteria).*

Stage I and Stage II:

- Patients with Acute Promyelocytic Leukemia (APL) as diagnosed by morphologic criteria, flow cytometric characteristics, and rapid cytogenetics or FISH or molecular testing
- Any previous treatment with vosaroxin
- Concomitant chemotherapy, radiation therapy
  - For patients with hyperleukocytosis with > 50,000 blasts/uL; leukopheresis or hydroxyurea may be used prior to study drug administration for cytoreduction at the discretion of the treating physician. Hydroxyurea must be stopped 24 hours prior to initiation of protocol defined therapy.
- Active CNS leukemia
- Active, uncontrolled infection. Patients with infection under active treatment and controlled with antibiotics are eligible.
- Active, uncontrolled graft vs. host disease (GVHD) following allogeneic transplant for non-AML condition (e.g. MDS, lymphoid malignancy, aplastic anemia). Patients with GVHD controlled on stable doses of immunosuppressants are eligible.
- Known HIV seropositivity
- Any other medical, psychological, or social condition that may interfere with study participation or compliance, or compromise patient safety in the opinion of the investigator or medical monitor
- LVEF < 40% as measured by echocardiogram or MUGA
- Women who are pregnant or breastfeeding
- Renal insufficiency requiring hemodialysis or peritoneal dialysis

STUDY TREATMENT:

- Cytarabine (commercial supply)
  - administered as a continuous infusion with a dose of 100 mg/m2 daily x 7 days
- Vosaroxin (supplied by Sunesis)
  - Induction: 90 mg/m2 infusion on days 1, 4
  - Re-induction: 70 mg/m2 infusion on days 1, 4

PRIMARY ENDPOINT:

- Complete remission

STATISTICAL PLAN:

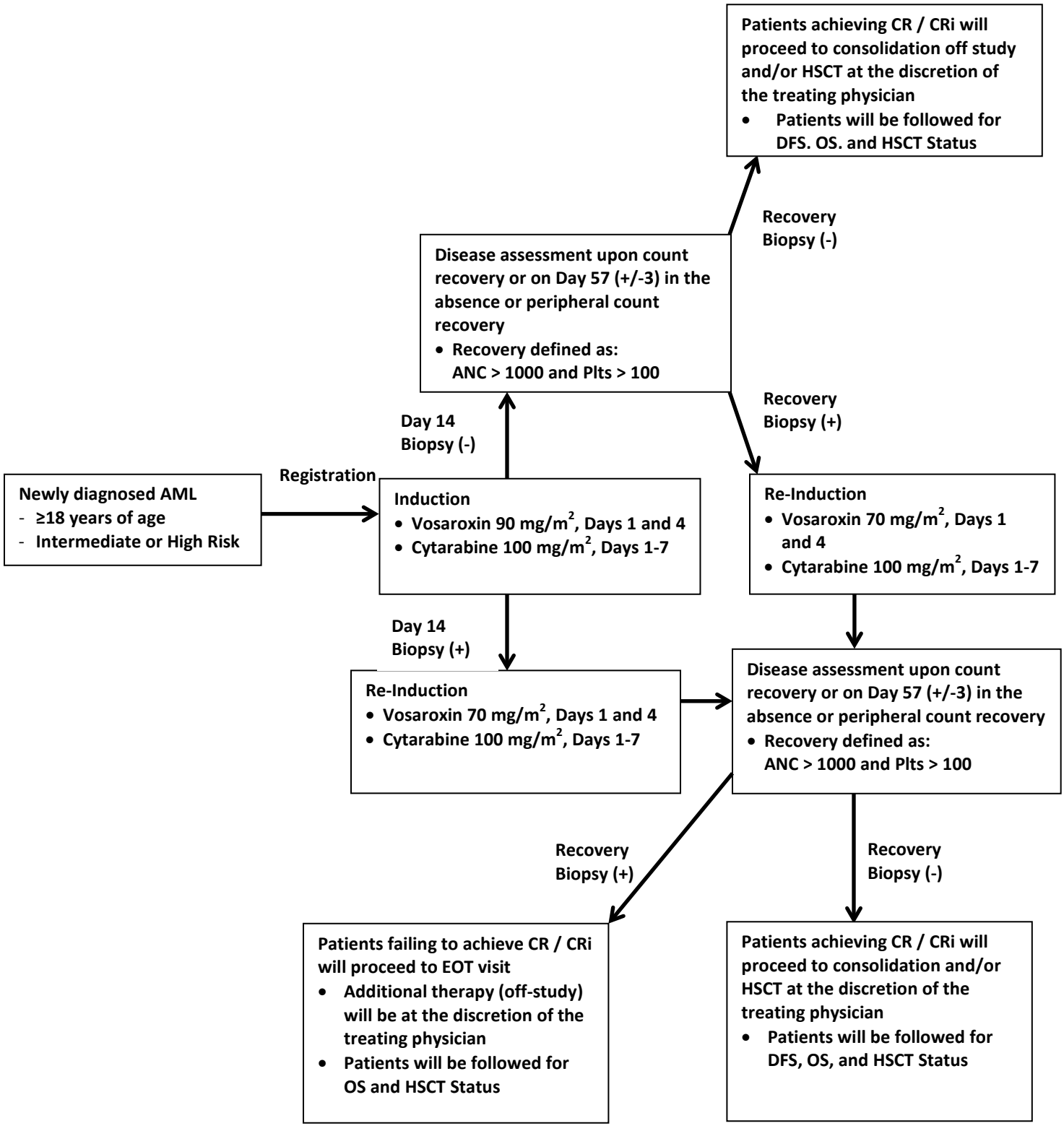
The primary objective of this single-arm, phase II clinical trial is to determine if vosaroxin in combination with standard dose cytarabine as induction chemotherapy can significantly improve the rate of complete remission (CR) in patients with untreated acute myeloid leukemia. Forty-one (41) patients will be enrolled in a two-stage design that permits one interim look to examine evidence of futility. As per convention, the study will not be stopped early on the basis of preliminary evidence of efficacy observed at the end of the first stage (i.e., at the interim look).

Ethical imperatives and resource constraints encourage the exploration of more adaptive designs. Hence we propose, in the context of a standard two-stage design, that a likelihood-based assessment of the statistical evidence be used. An important advantage is that, unlike p-values, which are binary in nature, likelihood ratios have three evidential zones: evidence favoring the null hypothesis, evidence favoring the alternative hypothesis, and weak or inconclusive evidence (i.e., favoring neither hypothesis).

In accordance with our desire to maintain a uniform study design, we set a total sample size of 41 participants. An interim futility analysis will be conducted following enrollment of 17 evaluable patients. If ≤ 7 patients achieve CR then the likelihood ratio in support of reaching the goal endpoint of $\geq 60\%$ remission would be diminished. In this case, the DSMB will review the clinical data to determine the merits of continued enrollment. If > 7 CRs are experienced by the first 17 evaluable patients, enrollment will automatically proceed to enroll a total of 41 patients. Under this two-stage design, a 60% remission endpoint by the "7+V" combination is 3.45 times more likely than a 40% response rate if 22 or more patients experience CR.

In the context of the planned likelihood assessment the option will be provided to add an extension cohort of up to 20 participants if the initial trial results are inconclusive. In this scenario, the use of a likelihood based extension cohort results in false positive and false discovery rates of 3.7% and 3.9%, respectively.

PROTOCOL SCHEMA



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1. BACKGROUND

1.1. Acute Myeloid Leukemia

Data from the American Cancer Society estimate 18,860 new diagnoses of acute myeloid leukemia in the United States in 2014 and an alarming 10,460 deaths of patients with this diagnosis.[1] Although there has been no major change in induction therapy strategy for nearly 4 decades[2], subgroups that respond particularly well or poorly have been identified based on their genetic features.[3-7] Careful examination of disease characteristics at diagnosis is imperative to risk-stratify patients and appropriately apply novel risk-adapted therapies or intensified treatment strategies including hematopoietic stem cell transplantation (HSCT).

1.2. Study Rationale

AML is an aggressive and highly fatal disease with a median age of 67 years at diagnosis and an unfortunate age-adjusted 5-year survival of 24.2%.[8] Given the advanced age of the majority of patients with this diagnosis and/or the medical comorbidities often associated with their advanced age, many AML patients are not offered the conventional intensive strategy of "7+3" (cytarabine + anthracycline). Many older patients who are deemed medically appropriate to receive an intensive induction strategy tend to have AML with poor risk cytogenetic or molecular features and only ~40% are expected to achieve complete remission.[9,10] Because of these features, the 5-year overall survival of patients above the age of 55 approaches only 10%.[9] While most patients with newly diagnosed AML are over 55 years of age, adult patients aged 18-55 do develop AML, and have variable success with standard therapy of 7+3. With the exception of favorable risk AML in younger patients, the 5 year survival for patients less than 55 years of age remains less than 40%.[6] The poor long-term survival of these patients combined with a lack of available novel therapeutic strategies in the past 40 years warrants the investigation of newer agents in the frontline setting.

As vosaroxin ± cytarabine has been successfully administered and tolerated in both AML patients of advanced age (≥ 60 years)[11] and all patients with relapsed/refractory disease,[12,13] [14] we will pursue the investigation of the combination of cytarabine with vosaroxin ("7+V") in newly diagnosed AML patients.

There is an established, acceptable safety profile of infusional cytarabine 400mg/m^2 given over 5 days concomitantly with vosaroxin. Pharmacokinetic studies from this combination have established the rapid, linear elimination of vosaroxin in the presence of infusional cytarabine and provide precendent and rationale for vosaroxin with 7 days of infusional cytarabine given in the customary fashion at a dose of 100mg/m2/day. This combination will provide the opportunity to expand upon the previously observed efficacy of vosaroxin in patients with high-risk (relapsed/refractory) AML[14] and has the potential to

provide a foundation for a future randomized phase III trial comparing "7+V" to conventional "7+3".

1.3. Investigational Product

Vosaroxin is a first-in-class anticancer quinolone derivative (AQD) currently in clinical development by Sunesis Pharmaceuticals, Inc. as a treatment for acute myeloid leukemia (AML). The molecular formula of vosaroxin is $C_{18}H_{19}N_5O_4S$ and its molecular weight is 401.45 Daltons. Vosaroxin is administered as a short intravenous (IV) infusion within 10 minutes.

Vosaroxin has a unique mechanism of action distinct from other cytotoxic anticancer agents. The activity of vosaroxin in mammalian cells appears to parallel the activity of the quinolone antibiotics in prokaryotes.[15-17] Vosaroxin intercalates DNA and inhibits topoisomerase II activity through both enzymatic inhibition and poisoning.[18] This leads to site-selective DNA double-strand breaks (DSB) in G/C-rich sequences, which are characteristic sites of quinolone-induced DNA cleavage.[19] Vosaroxin targets actively replicating cells, and the extent of DNA damage is cell-cycle dependent, with the damage induced in G2/M > S >> G1. The DNA damage results in a delay in S phase, arrest at G2/M phase, and cell death by apoptosis. Vosaroxin is minimally metabolized and does not require metabolism for cytotoxic activity.

Vosaroxin and cytarabine have non-overlapping primary molecular mechanisms of action.[20] In contrast to vosaroxin, cytarabine is a pyrimidine nucleoside analog that requires cellular uptake and metabolism to form the active metabolite, arabinosyl-cytosine triphosphate (ara-CTP). The activities of ara-CTP, namely inhibition of DNA polymerase and DNA ligase, and direct incorporation into DNA, result in DNA damage and termination of synthesis. Cytarabine activity is highly specific for the S phase of the cell cycle.

Phase 1 Studies SPO-0001 & SPO-0002: Relapsed/Refractory Solid Tumors

The purpose of these studies was to assess the vosaroxin dose-limiting toxicity (DLT), maximum-tolerated dose (MTD), pharmacokinetics and clinical activity of vosaroxin in patients with relapsed/refractory solid tumors. [21] Two dose-escalation studies evaluated IV vosaroxin administered every 3 weeks (SPO-0001) or weekly every 28 days for 3 weeks (SPO-0002). In SPO-0001, patients were classified as heavily pretreated (HP) or minimally pretreated (MP) based on therapeutic history.

In the SPO-0001 study (N=41; 24 HP/17 MP), patients were treated in eight dose cohorts using 3-75 mg/m^2. At the 60 mg/m^2 dose, four HP patients experienced DLTs of grade 4 neutropenia (N= 3; one with fever) and grade 3 febrile neutropenia/pneumonia (N=1). At the

75 mg/m^2 dose, two MP patients experienced DLTs of grade 4 neutropenia/thrombocytopenia (N=1) or grade 2 oral thrush for >29 days (N=1). The MTD was established at 48 mg/m^2 (HP patients) and 60 mg/m^2 (MP patients).

In the SPO-0002 study (N=21), patients were treated in six dose cohorts using 3-24 mg/m^2. At the 18 mg/m^2 dose, two patients experienced DLTs of grade 3 neutropenia (one with pleural effusion, each >14 days). The MTD was established at 15 mg/m^2. Vosaroxin exhibited low clearance (2 L/h/m^2), a long terminal half-life (22 hours), and dose-proportional exposure. Overall, 31 of 62 patients achieved stable disease and one patient (ovarian cancer) achieved a partial response per Rustin criteria.

Phase 1b SPO-0004 Trial: Relapsed/Refractory Leukemia

The purpose of this study was to evaluate the DLT, MTD, pharmacokinetics (PK), clinical activity and pharmacodynamics of vosaroxin in patients with in relapsed/refractory leukemia (N=73). [12] Vosaroxin was administered weekly on days 1, 8 and 15 or twice weekly on days 1, 4, 8 and 11. The treated patients had a median age of 65 years, 85% had acute myeloid leukemia and 78% had refractory disease. Patients treated on the weekly schedule (N=42) received 18-90 mg/m^2 and the MTD was found to be 72 mg/m^2. Patients treated on the twice-weekly schedule (N=31) received 9-50 mg/m2 and the MTD was 40 mg/m2. The DLT was stomatitis and the primary non-hematologic toxicity was reversible gastrointestinal symptoms and febrile neutropenia. The 30-day all-cause mortality was 11%. Five patients had complete or incomplete remissions with a median duration of 3.1 months. A morphologic leukemia-free state (bone marrow blast reduced to <5%) occurred in 11 additional patients. Antileukemic activity was associated with total dose or weekly time above 1 μmol/L plasma vosaroxin concentration ($p<0.05$). Vosaroxin exposure was dose proportional over 9-90 mg/m2 dose range The average terminal half- life was ~25 h and clearance was non-renal. No induction or inhibition of vosaroxin metabolism was evident. Vosaroxin-induced DNA damage was detected as increased intracellular γH2AX.

Phase 1/2 SPO-0012 Trial: Relapsed/Refractory AML

This study assessed the safety and tolerability of vosaroxin plus cytarabine in patients with relapsed/refractory (AML). 13,22 Escalating vosaroxin doses (10-90 mg/m2; Days 1 and 4) were given in combination with cytarabine using one of two schedules: Schedule A (24-hour continuous IV infusion, 400 mg/m2/day, Days 1-5) or Schedule B (2-hour IV infusion, 1 g/m2/day, Days 1 and 5). Following dose escalation, enrollment was expanded at the MTD. Among the 110 patients enrolled, 108 received treatment. The MTD for schedule A was vosaroxin 80 mg/m2 (dose-limiting toxicities: grade 3 bowel obstruction and stomatitis); the MTD was not reached for schedule B (recommended phase 2 dose: 90 mg/m2). In the efficacy population (all first relapsed or primary refractory patients treated with vosaroxin 80-90 mg/m2; N=69), the complete

remission (CR) and combined CR (CR or CR with incomplete blood count recovery) rates were 25% and 28%, respectively. The 30-day all-cause mortality was 2.5% among all patients treated at 80-90 mg/m2.

Phase 2 SPO-0014 Trial: Previously Untreated AML

This study evaluated single-agent vosaroxin in patients ≥60 years of age with previously untreated AML with an unfavorable prognosis (N=113). [23,24] Dose regimen optimization was explored in sequential cohorts (A: 72 mg/m2 days 1, 8, 15; B: 72 mg/m2 days 1, 8; C: 72 mg/m2 or 90 mg/m2 days 1, 4). The primary endpoint was combined complete remission (CCR) rate (complete remission [CR] plus CR with incomplete platelet recovery). Common (>20%) grade ≥3 adverse events were thrombocytopenia, febrile neutropenia, anemia, neutropenia, sepsis, pneumonia, stomatitis, and hypokalemia. Overall CR and CCR rates were 29% and 32%; median overall survival (OS) was 7.0 months; 1-year OS was 34%. Schedule C (72 mg/m2) had the most favorable safety and efficacy profile, with faster hematologic recovery (median 27 days) and lowest incidence of aggregate sepsis (24%) and 30-day (7%) and 60-day (17%) all-cause mortality. At this dose and schedule, CR and CCR rates were 31% and 35%, median OS was 7.7 months, and 1-year OS was 38%. Overall, vosaroxin resulted in low early mortality and an encouraging response rate; vosaroxin 72 mg/m2 days 1, 4 is recommended for further study in this population.

Phase 1/2 MD Anderson Cancer Center Trial: Older patients with Newly Diagnosed AML and High Risk MDS

Patients eligible for enrollment in this study had AML or high-risk MDS (defined as having ≥ 10% blasts in the bone marrow), were ≥60 years old and had adequate performance status (ECOG ≤ 2) and organ function. [25] Patients <60 who were unsuitable for standard chemotherapy were also eligible. During phase 1 of the study, the first six patients received vosaroxin 90 mg/m2 daily on days 1 and 4 with decitabine 20 mg/m2 daily for 5 days. This dose was well-tolerated and used for phase 2 of the study; however, the induction dose was reduced to 70 mg /m2 starting with patient 25 due to mucositis. The vosaroxin dose could be maintained at 70 mg/m2 or reduced to 50 mg/m2 in consolidation cycles, which were repeated in approximately 4 to 5-week intervals for a total of up to 7 cycles of vosaroxin and decitabine. Dose adjustments and dose delays of one or both agents, were allowed based on toxicity.

The primary endpoint was the overall response rate including complete response (CR) + CR without platelet recovery (CRp) + CR with insufficient hematological recovery (Cri). Secondary endpoints were: CR duration, disease-free survival, overall survival, safety, and early mortality. At the time of this report, 34 patients with AML (N=31) and high-risk MDS (N=3) with a median age of 70 years (range, 41-78) had been enrolled and 33 (97%) were >60 years old. These included 14 (41%) with diploid cytogenetics, 12 (35%) with complex

cytogenetic abnormalities including chromosome 5 and/or 7 abnormalities, and eight (24%) with other miscellaneous abnormalities. Twelve patients with AML (36%) had antecedent hematological disorders (AHD) including MDS (N=7; 21%), myeloproliferative neoplasm (N=3; 9%) and MDS/MPN (N=2; 6%). Three patients with AHD had received prior therapy including 5-azacytidine (N=1), ruxolitinib + 5- azacytidine (N=1) and lenalidomide (N=1). Additionally, five patients (15%) had therapy- related disease with prior exposure to chemotherapy or radiation therapy. Median bone marrow blast%, median white blood cell, hemoglobin, and platelet counts were 38% (range, 9-97), 4.1 x 10^9/L (range, 0.4-57.0), 9.4 g/dL (range, 6.8-11.5), and 41 x 10^9/L (range, 7-333), respectively. The patients evaluable for response (N=30) achieved CR (N=13; 43%), CRp (N=6; 20%), and CRi (N=3; 10%) for an overall response rate of 73%.. Patients had received a median of 2 (range, 1-6) treatment cycles with the median number of cycles to response being 1 (range, 1-4). The median duration of CR/CRp/CRi had not been reached. Four patients had proceeded to allogeneic stem cell transplant. The 4- and 8-week mortality rates were 0% and 17%, respectively. The regimen was well tolerated with the main grade ≥ 3 toxicity being mucositis (N=8; 24%).

Phase 3, randomized, double-blind, placebo-controlled, pivotal clinical trial of vosaroxin in combination with cytarabine to evaluate overall survival in patients with first relapsed or refractory AML

Patients eligible for this trial were > 18 years of age with either refractory AML (persistent disease after induction, or first complete remission [CR1] < 90 d) or AML in first relapse (early relapse: CR1 of 90 d to 12 mo; late relapse: CR1 of 12 mo to 24 mo). Patients were allowed to have received 1-2 cycles of prior induction chemotherapy including at least 1 cycle of prior anthracycline (or anthracenedione) and cytarabine. Randomization was stratified by disease status (refractory, early relapse, late relapse), age (< 60, ≥ 60 years), and geographic location (US, non-US). Primary efficacy and safety endpoints were overall survival (OS) and 30- and 60-day mortality; secondary endpoints were complete remission (CR) rate and incidence of adverse events (AEs).

Enrollment occurred between December 2010 and September 2013 with 711patients randomized to receive either vosaroxin+cytarabine (n = 356) or placebo+cytarabine (n = 355) at 124 sites. Per the adaptive design, a prespecified 1-time sample size increase of 225 patients was implemented after the interim analysis. At the final analysis, median OS was 7.5 mo (95% CI: 6.4-8.5) with vosaroxin+cytarabine vs. 6.1 mo (95% CI: 5.2-7.1) with pla/cyt (HR = 0.866 [95% CI: 0.73-1.02]; 2-sided unstratified log-rank P = 0.06). The OS difference was statistically significant in a preplanned analysis accounting for the stratification factors at randomization (2-sided stratified log-rank P = 0.02).

Overall, 29.5% of patients underwent allogeneic stem cell transplant, including 45.8% of patients < 60 years and 20.2% of patients ≥ 60 years. Transplant rates were comparable between the 2 treatment arms (30.1% with vosaroxin+cytarabine and 29.0% with

placebo/cytarabine). In a predefined analysis censoring for subsequent ASCT, median OS was improved with vosaroxin+cytarabine (6.7 mo vs 5.3 mo with placebo+cytarabine; HR = 0.81 [95% CI: 0.67-0.97]; *P* = 0.02; stratified *P* = 0.03). In predefined subgroup analyses, OS benefit was greatest in patients aged ≥ 60 years (7.1 mo with vosaroxin+cytarabine vs 5.0 mo with placebo+cytarabine; HR = 0.75; *P* = 0.003) and those with early relapse (6.7 mo vs 5.2 mo; HR = 0.77; *P* = 0.04). OS with vosaroxin+cytarabine vs. placebo+cytarabine was 9.1 mo vs 7.9 mo in patients < 60 years (HR = 1.08; *P* = 0.60); 6.7 mo vs 5.0 mo in patients with refractory disease (HR = 0.87; *P* = 0.23); and 14.1 mo vs 12.3 mo in patients with late relapse (HR = 0.98; *P* = 0.96), respectively. A CR was achieved in 30.1% of patients treated with vosaroxin+cytarabine vs. 16.3% treated with placebo+cytarabine (*P* = 0.00001).

Thirty-day and 60-day all-cause mortality was similar in the 2 arms (30-day: 7.9% vs 6.6%; 60-day: 19.7% vs 19.4% with vosaroxin+cytarabine vs. placebo+cytarabine, respectively). Most common serious AEs were febrile neutropenia (11.3% with vosaroxin+cytarabine 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%). Serious and non-serious cardiac, renal, neurologic, and hepatic AEs were comparable between treatment groups.

2. OBJECTIVES

2.1. Primary Objectives

- To assess the rate of complete remission (CR) after induction therapy with the combination of "7+V" [vosaroxin and standard dose infusional cytarabine] for patients with newly diagnosed, previously untreated acute myelogenous leukemia (AML)

2.2. Secondary Objectives

- To further evaluate and the safety and tolerability of "7+V"
- To evaluate for the presence of minimal residual disease (MRD) remaining after "7+V" induction and/or re-induction
- To determine CR/ CRi rate after one and / or 2 cycles of "7+V" induction
- To determine time to neutrophil and platelet recovery following "7+V" induction
- To assess disease free survival (DFS) at 1yr of patients achieving CR / CRi after "7+V" induction
- To assess overall survival (OS) at 1yr of all patients receiving protocol defined therapy
- To determine correlation of pretreatment HSCT comorbidity index, AML-Score, and Wheatley Index scores with disease response, DFS and OS

2.3. Exploratory Objectives

- To describe the mutational burden of this cohort of AML patients
- To correlate genomic aberration with response rate, DFS, and OS
- To determine the number of patients treated with vosaroxin who eventually go to allogeneic HSCT

3. STUDY DESIGN

3.1. Overall Design and Plan

- Single-arm, open-label, phase II study of vosaroxin in combination with cytarabine in patients with newly diagnosed AML.
- Vosaroxin will be administered intravenously at 90 mg/m2 on days 1 and 4. Cytarabine will be administered in standard fashion as a continuous infusion of 100 mg/m2 daily on days 1-7.
  - Patients with evidence of residual leukemia on "Day 14 biopsy" following initial induction will be offered re-induction with intravenous vosaroxin at 70 mg/m2 on days 1 and 4 in combination with continuous infusion cytarabine at 100 mg/m2 daily on days 1-7.
- Two-stage design. Forty-one (41) patients will provide 80% power to detect a 20% increase in complete remission rates (from 40% to 60%) with a Type I Error rate of 5%. The design permits one interim look to examine evidence of futility after the first 17 patients are evaluable for response. If ≤ 7 patients achieve CR then the likelihood of reaching the goal endpoint of $\geq 60\%$ remission would be diminished. In this case, the DSMB (consisting of a representative from the funding organization, the study chairs, a biostatistician, and participating investigator to be selected by the study chairs) will review the clinical data to determine the merits of continued enrollment. If > 7 CRs are observed then the second stage will open automatically and increase enrollment to 41 patients.
  - Stage 1: First 17 patients who are evaluable for response prior to futility analysis
  - Stage 2: Patients 18-41 enrolled after futility analysis
- Likelihood-based assessment of the statistical evidence be used to determine the reliability of the data generated and will provide the option of adding one or two extension cohorts of 10 participants each if the initial trial results are inconclusive.

3.2. Duration of Study

- First potential date of Study Activation: Feb 01, 2016
- Accrual duration: 12 months
- First patient enrolled/treated: Mar 2016
- Last patient enrolled/treated: Feb 2017
- Completion of 12-month follow-up post enrollment: Feb 2018

4. SELECTION OF STUDY POPULATION

4.1. Inclusion Criteria

Patients meeting the following criteria will be eligible:

- Age ≥ 18 years of age
- Ability to provide informed consent
- Ability to tolerate intensive therapy with vosaroxin 90mg/m2 and standard dose of cytarabine as per investigator discretion
- ECOG performance status 0-2 at time of study entry (Appendix A)
- Morphologically confirmed new diagnosis of AML in accordance with WHO diagnostic criteria (Appendix B)
- Patients who have received hydroxyurea alone or have previously received "non-cytotoxic" therapies for MDS or MPN (e.g., thalidomide or lenalidomide, 5-azacytidine or decitabine, histone deacetylase inhibitors, low-dose cytoxan, tyrosine kinase or dual TK/src inhibitors) will be allowed
- Renal function: Serum creatinine ≤ 2.0 mg/dL
- Hepatic enzymes (ALT, AST) ≤ 2.5 x upper limit of normal
- Total bilirubin ≤ 1.5 x upper limit of normal unless clearly related to Gilbert's Disease, hemolysis or leukemic infiltrate

- For patients in **stage 1 (patients #1-#17)**.
  - ≥ 55 years of age with AML of any risk classification, OR
  - 18-54 years of age with high-risk AML disease based on one of the following:
    - Antecedent hematologic disorder including myelodysplasia (MDS)-related AML (MDS/AML) and prior myeloproliferative disorder (MPD)
    - Treatment-related myeloid neoplasms (t-AML/t-MDS)
    - AML with FLT3-ITD
    - Myeloid sarcoma
    - AML with multilineage dysplasia (AML-MLD)
    - Adverse cytogenetics (defined as -5/-5q; -7/-7q; abnormal 3q, 9q, 11q, 20q, 21q or 17p; t(6;9); t(9;22); trisomy 8; trisomy 13; trisomy 21; complex karyotypes (≥ 3 clonal abnormalities); monosomal karyotypes

- For patients in **stage 2 (enrolled patient #18 and beyond)**.
  - ≥ 55 years of age with AML of any risk classification, OR
  - 18-54 years of age with intermediate or high risk AML as defined by NCCN risk assignment (Appendix C)

4.2 EXCLUSION CRITERIA:

Stage I:

- Patients 18-54 years of age with "good risk" AML defined as the presence of t(8;21) , inv(16), or t(16;16) as diagnosed by morphologic criteria, flow cytometric characteristics, and rapid cytogenetics or FISH *(Patients with t(8;21), inv(16), t(16;16) who are unable to receive anthracycline based induction will be allowed to enroll provided the medical reason they are unable to receive anthracyclines is clearly documented and provided they fulfill all other eligibility and criteria).*

Stage I and Stage II:

- Patients with Acute Promyelocytic Leukemia (APL) as diagnosed by morphologic criteria, flow cytometric characteristics, and rapid cytogenetics or FISH or molecular testing
- Any previous treatment with vosaroxin
- Concomitant chemotherapy, radiation therapy
  - For patients with hyperleukocytosis with > 50,000 blasts/uL; leukopheresis or hydroxyurea may be used prior to study drug administration for cytoreduction at the discretion of the treating physician. Hydroxyurea must be stopped 24 hours prior to initiation of protocol defined therapy.
- Active CNS leukemia
- Active, uncontrolled infection. Patients with infection under active treatment and controlled with antibiotics are eligible.
- Active, uncontrolled graft vs. host disease (GVHD) following allogeneic transplant for non-AML condition (e.g. MDS, lymphoid malignancy, aplastic anemia). Patients with GVHD controlled on stable doses of immunosuppressants are eligible.
- Known HIV seropositivity
- Any other medical, psychological, or social condition that may interfere with study participation or compliance, or compromise patient safety in the opinion of the investigator or medical monitor
- LVEF < 40% as measured by echocardiogram or MUGA
- Women who are pregnant or breastfeeding
- Renal insufficiency requiring hemodialysis or peritoneal dialysis

5. STUDY PROCEDURES

(see Table 1: Study Calendar)

5.1. Screening

Eligibility requirements and signed informed consent should be obtained within 15 days prior to Induction 1, Day 1.

5.1.1 TRIAL REGISTRATION

The Vanderbilt-Ingram Cancer Center (VICC) Multi-Institutional Coordinating Center (hereafter referred to the as "the Coordinating Center") will coordinate enrollment onto the study. Once the Coordinating Center confirms eligibility and approves enrollment onto the study, it will provide the sites with confirmation and assigned patient ID numbers.

5.1.2 TRIAL REGISTRATION PROCESS

To enroll a patient onto the study, participating sites should submit the enrollment packet to the Coordinating Center for eligibility confirmation and enrollment prior to the initiation of protocol therapy.

The enrollment packet includes:

- Enrollment Form
- Eligibility Screening Worksheet
- Signed patient consent form
- Eligibility supporting documents such as pathology reports, laboratory tests, etc.

The enrollment packet should be submitted via secure email transfer or fax using the contact information listed below:

Attn: VICC Multi-Institutional Coordinating Center

Fax: 615-875-0040
or
Coordinating.Center@vanderbilt.edu

The enrollment packet should be submitted to the Coordinating Center as early as possible to ensure timely patient registration. Same-day enrollments cannot be guaranteed.

Vanderbilt typically does not grant exceptions to eligibility criteria.

Once the Coordinating Center has confirmed eligibility, the site will be notified via email with assigned patient sequence number.

5.2. Treatment / Hematologic Recovery

The treatment period will include up to two cycles of treatment (Induction 1, Induction 2). In each cycle, the first day of study treatment will be considered day 1. Each cycle will include vosaroxin treatment on Days 1 and 4 and cytarabine on Days 1-7 followed by a variable interval required to achieve hematologic recovery defined as absolute neutrophil count (ANC) > 1000 cells/μL and platelets > 100,000 cells/μL. Hematologic recovery is expected to occur approximately 5 to 6 weeks after the first treatment day of each cycle. Each cycle will be a minimum of 14 days and a maximum of 8 weeks.

5.2.1. Induction 1

An early assessment of antileukemic activity (bone marrow biopsy or aspirate) will be performed at approximately Day 14 for the sole purpose of guiding treatment decisions. If the early treatment assessment indicates an ablated bone marrow with < 5% blasts, CBC will be examined as noted in the schedule of events thereafter to monitor for hematologic recovery.

If the early treatment assessment indicates residual leukemia (≥ 5% blasts) and a second cycle is indicated in the judgment of the investigator, the investigator may initiate Induction 2 any date prior to Induction 1 day 57 (no later than 8 weeks after Induction 1, Day 1) if eligibility criteria are met. Bone marrow assessments may be repeated as necessary per standard of care (investigator discretion) to clarify or confirm a finding of residual leukemia. In the event that blasts are ≥ 5% blasts, but ≤ 10%, the investigator may choose to pursue an additional bone marrow biopsy at a future date prior to making decision on whether Induction 2 is warranted.

5.2.2 Induction 2

Induction 2 will not be necessary for all patients, but may be appropriate if the Induction 1 early treatment or hematologic recovery assessment indicates residual leukemia is present, and if a second cycle is indicated in the judgment of the investigator. Eligibility for Induction 2 requires that non-hematologic toxicity and abnormal laboratory values be resolved to grade ≤ 1, if assessed by the investigator as related to treatment and clinically significant. Recovery of ANC, hemoglobin/hematocrit, and/or platelet counts is not required prior to Induction 2.

5.2.3 Consolidation

Consolidation therapy is not defined by the protocol. Patients achieving CR/CRi will proceed with consolidation therapy and/or hematopoietic stem cell transplantation at the discretion of the treating physician in accordance with institutional practice.

5.3. End of Treatment

End of treatment procedures will be performed for all patients when study treatment is discontinued for any reason or another AML therapy is started, including conditioning regimens for transplantation. Every reasonable effort will be made to complete the procedures to prevent loss to follow up. A separate visit may not be required, and the procedures performed at the last visit may satisfy the required end of treatment procedures. Ongoing serious adverse events (SAEs) assessed by investigators as related to study treatment will be followed with reasonable effort until the event resolves, stabilizes, is considered irreversible, or the patient receives a new AML therapy or dies.

5.4. Follow-Up

At the end of treatment, the purpose of follow-up will be to document survival status for all patients and to document disease status for patients in continuing CR or CRi. Every reasonable effort must be made to obtain information about patient status. In the event of death, additional information will be obtained, such as the date of death, cause of death, date of last contact, and laboratory and bone marrow results to document disease status at the time of death, such as continuing remission, relapse, persistent or recurrent leukemia. Follow-up for all patients will be based on chart review and/or phone call every 3 months after completion of protocol defined therapy for one year.

TABLE 1. Study Calendar

Procedures	Screening	C1: induction		C1: response assessment	C2: induction			C2: response assessment	EoT [p]
	Within 15 days of C1D1	D1	D4	Weekly until recovery or D57 (+/- 3d) [j,k,l,m]	Within 3 days of C2D1	D1	D4	Weekly until recovery or D57 (+/-3d) [j,k,l,m]	
TESTS & OBSERVATIONS									
Informed consent [a]	X								
Vital signs [b]	X	X	X	X		X	X	X	
H&P [b]	X	X				X			
ECOG PS [b]	X	X		X		X		X	
Directed physical exam [b]			X	X	X		X	X	
Adverse event evaluation [c]		X	X	X	X	X	X	X	
Concomitant med review	X	X	X	X		X	X	X	
Vosaroxin [d]		X	X			X	X		
Cytarabine [d]		X				X			
LABS									
Echocardiogram [e]	X				X				
EKG [f]	X		X		X		X		
CBC with differential [g]	X	X	X	X		X	X	X	X
Blood chemistry [h]	X	X	X	X		X	X	X	
PT, PTT, INR, fibrinogen	X								
Serum pregnancy test [i]	X								
DISEASE ASSESSMENT									
Bone marrow aspirate and core [j]	X			X [o]				X	X
Molecular, cytogenetics, FISH [k]	X			X				X	X
Correlative laboratory studies [l]	X			X				X	X
Disease status/survival [m]				X				X	X
Comorbidities [n]	X								

Long Term Observations	**Follow-up via telephone and/or chart review**	
	6mo after diagnosis	12mo after diagnosis
Disease Status/Survival[m]	X	X
Allogeneic HSCT status	X	X

Schedule of Assessments for VITAL STUDY

[a] Informed consent must be obtained within 15 days prior to initiation of trial treatment

[b] The screening physical examination, ECOG performance status, hematology, chemistry, PT/INR and serum pregnancy test must be conducted within 7 days from C1D1. If the initial examinations are obtained within 72 hours of C1D1, they do not need be repeated. At the baseline visit only, height will also be recorded. PEs and laboratory assessments will be

completed until count recovery as directed on calendar. Directed physical exams as per investigator discretion. Vital signs to include weight.

[c] Adverse event reporting will be required on days as noted in schedule of assessments. In the event that AEs occur between these visits, they will be captured if they meet criteria as defined in section 10.

[d] Vosaroxin is administered on D1 and D4 as per section 3.1. Oral cryotherapy will be utilized in association with Vosaroxin administration (see appendix E). Cytarabine will be administered via continuous infusion D1-D7.

[e] Echocardiogram will be conducted to assess cardiac function prior to administration of chemotherapy. In the event that there are abnormalities on the initial echocardiogram, or if the investigator has concern for cardiac complications during the first induction, a second echocardiogram may be conducted as per standard of practice.

[f] EKGs will be obtained during screening and again after vosaroxin administration on D4 visit.

[g] CBC with differential will include RBC, Hgb, hematocrit, total WBC, ANC, platelet count, and ≥ 5 part differential

[h] Chemistry panel includes the following: urea or BUN, creatinine, sodium, potassium, calcium, magnesium, phosphorus, glucose albumin, total protein, ALT, AST, total bilirubin, uric acid, and LDH

[i] A serum pregnancy will be performed only at baseline when appropriate as per protocol.

[j] Bone marrow biopsy and aspirate are required prior to enrollment. In the event that a diagnostic bone marrow aspirate/biopsy was performed prior to study-specific informed consent being obtained, diagnostics from this marrowing may be reported (and specimen collection at screening will be limited to biospecimens for lab correlates, see footnote L below). Bone marrow aspirate and biopsy also are required at the following time points:

- **C1D14 (+/- 2 days)**: for assessment of residual leukemia. Patients with residual leukemia at this assessment are eligible for a second round of induction (C2).
- **During *response assessment period* (C1 and/or C2)**: upon peripheral count recovery (as determined per weekly assessments); **OR** per investigator discretion, even in absence of count recovery; **OR** at EoT occurring no later than D57 (e.g., in absence of peripheral count recovery, or patient still being followed for inconclusive prior marrow). Note that marrow biopsy/aspirate is **NOT** required to be repeated as an EoT activity if already performed at recovery.

[k] Molecular (mutation testing) and cytogenetic (classic karyotyping testing ± FISH) will be performed as per standard of care. These analyses should be reported at study enrollment and in setting of response assessment at recovery or EoT, per standard of care.

[l] Peripheral blood and aspirate from bone marrow will be required as per the laboratory manual at study enrollment and in setting of response assessment at recovery or EoT.

[m] Disease assessment and survival will be noted during therapy and at 6 and 12mo follow up, as per section 3.1. During therapy, disease assessment should be reported in setting of response assessment at recovery or EoT.

[n] Co-morbidities and risk stratification will be assessed prior to therapy via HCT-Comorbidity index [26], Wheatley index [27], AML-SCORE [28] (see Appendix F), and then correlated with response to induction and rates of allogeneic hematopoietic stem cell transplant as per exploratory endpoints.

[o] At C1D14 (+/- 2 days), bone marrow required for assessment of residual leukemia. Otherwise, disease assessment procedures during *response assessment period* should be performed per footnotes J, K, L, and M.

[p] EoT activities required ONLY if disease assessment procedures not already performed in setting of count recovery during *response assessment period* (C1 and/or C2). See also footnote J, bullet point 2.

6. STUDY DRUG ADMINISTRATION

6.1. Vosaroxin Formulation

Each vial contains 2,300 mg vosaroxin at a concentration of 10 mg/mL. Each milliliter also contains 45 mg of D-sorbitol to maintain isotonicity. Methanesulfonic acid is added as an aid to solubilize vosaroxin. The sterile nonpyrogenic solution is formulated without preservatives.

6.2. Vosaroxin Storage

Vosaroxin vials must be stored as packaged at controlled room temperature, thermostatically maintained from 20°C to 25°C (68°F to 77°F). Do not store above 25°C. Stability data supports variations in prevailing temperature between 15°C and 30°C. Vosaroxin must not be frozen or stored in a refrigerator. When vosaroxin is drawn into a syringe, stability data support a maximum of 2 hours exposure to continuous ambient light. If the syringe containing the product is completely wrapped in foil or other protective covering from ultraviolet and visible light (e.g., amber bag, brown paper bag), this 2-hour stability maximum can be extended up to 24 hours.

6.3. Dose Calculation, Preparation, and Administration

6.3.1. Dose Calculation

The administration of study drugs (vosaroxin and cytarabine) requires calculation of body surface area (BSA) using the patient's actual, measured body weight and height. For patients with a BSA > 2.4 m2, dosing should be calculated using a maximum BSA of 2.4 m2. The Mosteller formula[29] is recommended to calculate BSA in m2; however, pharmacy personnel may calculate BSA according to institutional method.

Mosteller formula: BSA (m2) = $\sqrt{\frac{height\ (cm)\ x\ weight\ (kg)}{3600}}$

6.3.2 Preparation and Administration of Vosaroxin

Undiluted vosaroxin should be administered according to these general guidelines:

- Wear gloves and adhere to strict aseptic technique.
- Inspect the vial and confirm it is not cracked or damaged in any way.
- Inspect the solution and confirm it is not discolored and contains no visible particulates.
- Do not infuse vosaroxin in the same IV line (tubing) with sodium chloride solutions or other medications. The product may precipitate in sodium chloride solutions. If no alternative line is available, flushed saline thoroughly with 5% dextrose in water (D5W) before and after administration.

Suggested Procedure for Preparation

- Calculate the dose according to physician instructions.
- Attach an appropriate gauge needle to the tip of the sterile plastic syringe that will be used to administer vosaroxin.
- Withdraw the product from the vosaroxin vial into the syringe. Note: a vacuum may result as the product is withdrawn. Avoid or release the vacuum per institutional practice.

Please note that if using a vent needle to aid in drawing investigational product from the vial, the vial should remain upright, and the vent needle should only be inserted into the head space containing air. The needle used to withdraw product should be inserted as far as possible to the bottom of the vial to withdraw the required volume of drug.

If pressurizing a vial, do not use a vent needle. Add the equivalent dose volume of air to the vial headspace in the upright position, invert the vial, insert the syringe needle into the solution, and withdraw the required volume of drug.

- When the syringe contains the entire dose to be administered, cap the syringe and discard the needle.
- Protect the filled, capped syringe from light and whilst in transit to the patient until ready to administer the product.

Suggested Procedure for Administration

- A biluminal or triluminal catheter is recommended. Start an IV infusion through one of the lumens using D5W for analogous solution at a slow rate sufficient to keep the vein open.
- Before administration of vosaroxin, increase the D5W infusion rate to approximately 100 mL per hour and continue this rate during the vosaroxin infusion.
- Administer vosaroxin via syringe by assessing and available hub or stopcock.
- Administer vosaroxin as a short infusion within 10 minutes, either manually or using a syringe pump.
- At the end of the infusion, flushed the IV line with D5W per institutional standard.
- If a central catheter or heparin lock is in place, it may be capped according to the sites usual procedures.

  Vosaroxin is not known to be a thoracic and/or irritant. If extravasation is observed or suspected, treat according to standard institutional procedures.

7. CONCOMITANT MEDICATIONS

Concomitant medications are recommended as prophylaxis for nausea, vomiting, and infections, and Darvon for managing myelosuppression as shown in table 2.

Myelosuppression is expected in patients with AML due to underlying disease, as well as due to chemotherapy (such as vosaroxin and cytarabine), or both. Most patients will have neutropenia, thrombocytopenia, or both at study entry. Myelosuppression may be managed with growth factor support and blood transfusion according to institutional standard of care, American Society of Clinical Oncology (ASCO) Practice Guidelines, and NCCN Practice Guidelines.

Infections secondary to myelosuppression are common in patients with AML, and may be related to underlying disease, chemotherapy, or both. Therefore, the use of prophylactic antibiotics, antifungal agents, and antiviral agents is required. A regimen of levofloxacin (500 mg po daily), fluconazole (400 mg po daily), and valacyclovir (500 mg po q12h) is suggested with substitutions permitted as per institutional formulary. (See Appendix E)

Table 2. Instructions for the use of concomitant medications and therapies

Category of use	Medication	Comment on use	Restriction on use
Recommended	Prophylactic antibiotics, antifungal agents, and antiviral agents	Required (see Appendix E)	None
	Prophylactic antiemetic medications	According to standard of care at individual study site	None
	Mouth Care	Suggested regimen See Appendix E	See Appendix E
Allowed	Oral allopurinol or rasburicase	At investigator's discretion according to standard of care at individual study site	None
	Leukapheresis		Before induction 1 day 1 only
	Transfusion of red blood cells		None
	Transfusion of platelets		None
	Transfusion of granulocytes		None
	Erythropoietin or darbepoetin		None
	Any other medications for supportive care		Must be necessary for the patient's welfare and expected not to interfere with evaluation of study treatment
Prohibited/Restricted	Hydroxyurea or medications to reduce to blast count	May use transiently for reduction of cells if WBC >/= 50,000	Allowed only >24hours prior to initiation of protocol defined therapy
	Myeloid growth factors (e.g., sargramostim, filgrastim, pegfilgrastim)	May only use if patient's clinical status declines, and investigator uses as rescue sepsis, etc.	Restricted to neutropenic sepsis, bacteremia or other severe infection
	Investigational products (those not approved for any indication by the FDA or other regulatory authorities	None	Prohibited until end of protocol defined therapy
	Nonprotocol treatment for acute myeloid leukemia	None	Prohibited until end of protocol defined therapy

8. DISCONTINUATION OF TREATMENT

Treatment may be discontinued for a variety of reasons, including patient withdrawal, investigator decision, and reasons specified by the protocol.

8.1. Discontinuation Criteria for Individual Patients

8.1.1. Patient Withdrawal

Patients may voluntarily withdraw consent to participate in the clinical study at any time and without giving any reason. Their withdrawal will not jeopardize their relationship with their healthcare providers or affect their future care. Patients may also choose to withdraw from study treatment, but agree to remain in the study for follow up procedures.

8.1.2. Investigator Discontinuation of Patient

The investigator may exercise medical judgment to discontinue study treatment if clinically significant changes in clinical status or laboratory values are noted.

8.1.3. Criteria for Protocol-Defined Required Discontinuation of Treatment

The protocol requires discontinuation of study treatment for the following reasons:

- Maximum allowed treatment completed
- Failure to achieve a CR or CRi after 2 attempts (Induction 1 / 2)
- Disease relapse after a CR or CRi
- Clinically significant neutropenia or thrombocytopenia in the absence of residual leukemia that does not recover to grade ≤ 2 within 57 days after the first dose of vosaroxin in any cycle
- Clinically significant treatment related non-hematologic AE in the absence of residual leukemia that does not recover to grade ≤ 2 within 8 weeks after the first dose of vosaroxin in any cycle
- Any AE that is unacceptable or intolerable
- Intercurrent illness that prevents the next cycle of treatment
- Pregnancy
- Other non-protocol systemic AML therapy or prohibited medication initiated
- General or specific changes, including protocol violations, rendering the patient unsuitable for further study treatment

8.1.4. Protocol violations and deviations

Protocol violations are defined as significant departures from protocol required processes or procedures that affect patient safety or benefit potential, or confound assessments of safety or clinical activity. A protocol deviation is a departure from the protocol that does not meet the above criteria. Protocol violations or deviations may be grouped into the following classes:

- Enrollment criteria
- Study activities (missed evaluations or visits)
- Noncompliance with dose or schedule, including dose calculation, administration or discontinuation criteria
- Investigational product handling, including storage and accountability
- Informed consent and ethical issues

The Sponsor is responsible for implementing and maintaining quality assurance and quality control to ensure that studies are conducted according to the protocol, GCP, and all applicable regulatory requirements. A protocol deviation is any noncompliance with the protocol. Noncompliance can be on the part of the study participant, the Investigator, or the study site staff. All protocol deviations are required to be reported to the Sponsor and submitted to the IRB per institutional guidelines. Deviations to the protocol are not permitted except when necessary to eliminate an immediate hazard to study subjects.

9. EFFICACY ASSESSMENTS

9.1. Determination of Overall Survival

Survival information will be collected for all patients in the study.

9.2. Determination of Response

The investigator will determine response based on local laboratory findings to guide treatment decisions. Response will be determined based on IWG response criteria as summarized in Table 3.

9.3. Time-to-Event Endpoints

In addition to OS, time to event endpoints include EFS and LFS.

TABLE 3. Vosaroxin Response Criteria Based on International Working Group Criteria[30]

Category	Neutrophils (cells/µl)	Platelets (plt/µl)	Bone marrow blasts (%)	Other requirements
Complete remission (CR)	>1000	≥ 100,000	<5	• No Leukemic blasts in peripheral blood. • Transfusion independence, except for infection, bleeding, or medical/surgical conditions predisposing to bleeding. • No extra medullary disease
CR with incomplete count recovery (CRi)	≤1000	≥ 100,000	<5	• Meets criteria for CR except for neutrophils
	OR			
	>1000	< 100,000	<5	• Meets criteria for CR except for platelets
Partial Remission (PR)	>1000	≥ 100,000	Decrease of ≥ 50% to value between 5% and 25%	
Treatment Failure (TF)	Persistent acute myeloid leukemia in blood or bone marrow, or therapy fails to achieve a remission of any category, or death prior to response assessment			

10. SAFETY ASSESSMENTS

10.1. Safety Monitoring

10.1.1. Common Adverse Events Attributed to Vosaroxin

The safety profile of vosaroxin is consistent with the common pharmacologic effects of cytotoxic chemotherapeutic agents. Myelosupression (including associated fatal infections) and gastrointestinal (GI) toxicity (including nausea, diarrhea, and upper GI mucositis) are known risks associated with vosaroxin treatment. Oral mucositis (stomatitis) was the DLT in patients with hematologic malignancies treated with higher doses of vosaroxin: up to 40 mg/m^2 once weekly for 4 weeks (28-day cycle) or up to 90 mg/m^2 once weekly for 3 weeks (28 day cycle; up to 270 mg/m^2/cycle). In patients treated with vosaroxin, oral mucositis is dose limiting and grade 3 or 4 oral mucositis usually appears within 2 weeks after the first dose.

Per the most recent reference safety information (IB, Version 12), infections and febrile neutropenia were reported at a higher frequencies in patients with hematologic malignancies (39.7% and 36.9 of 648 patients respectively) than in patients with solid tumors (12.3% and 9.2% of 284 respectively). The GI toxicities of stomatitis and diarrhea were also reported at higher frequencies in patients with hematologic malignancies (46.8% and 47.7% respectively) than in

patients with solid tumors (11.3% and 17.3% respectively). For patients with hematologic malignancies who received vosaroxin (N = 648), the most common treatment-related AEs (with at least 20% incidence) include nausea (53.2%), diarrhea (47.7%), stomatitis (46.8%), febrile neutropenia (36.9%), decreased appetite (31.3%), vomiting (30.1%), anemia (29.0%), thrombocytopenia (28.5%), neutropenia (20.1%), and fatigue (21.0%). The only two grade 3 and 4 treatment-related AEs reported in at least 20% of all vosaroxin treated patients (N = 932) are febrile neutropenia (26.4%) and neutropenia (21.5).

For patients with AML treated with higher doses of vosaroxin, oral mucositis is dose limiting and grade 3 or 4 oral mucositis usually appears within 2 weeks after the first dose. Oral mucositis can be managed with standard supportive care (Appendix E). The incidence of combined grade 3 and 4 nausea and vomiting in vosaroxin studies is low (2.7% and 1.4% respectively).

In general, adverse reactions are manageable with standard and diligent supportive care. Myelosuppression and GI toxicities should be managed according to Appendix E within the protocol.

10.2. Overdose

An overdose is defined as administering at least 25% more than the protocol-specified dose (i.e., ≥125% of the protocol-specified dose). Overdoses should be reported to the Coordinating Center regardless if occurrence of adverse event within 24 business hours of discovery as instructed per section 11.6. The clinical manifestation (if any) of an overdose should be reported as an AE or SAE, as appropriate.

10.2.1. Pregnancy

During the course of the trial, all female patients of childbearing potential (the definition of "women of childbearing potential" is listed in Appendix D), must contact the treating investigator immediately if they suspect that they may be pregnant (a missed or late menstrual period should be reported to the treating investigator) after the first dose of study treatment until end-of-treatment procedures are performed.

Male patients that suspect they may have impregnated a partner must also notify the treating investigator immediately. If an investigator suspects that a patient may be pregnant prior to administration of trial drug(s), the trial drug(s) must be withheld until the result of the pregnancy test is confirmed.

If a pregnancy is confirmed, the patient must not receive any trial drug(s), and must be discontinued from the trial, and the investigator must notify the Coordinating Center as soon as possible. The Coordinating Center will report the pregnancy to a Sunesis representative.

Subjects and their partners with reproductive potential must agree to use effective contraceptive measures during the study and for 2 months after the last dose of study treatment.

See Appendix D for information regarding effective contraception measures.

11. ADVERSE EVENT DEFINITIONS AND REPORTING

11.1. Adverse Event Definitions According to ICH Guidelines and FDA Final Rule on Safety Reporting Requirements

Updated definitions according to both ICH and the FDA final rule are provided within Table 4.

Table 4. Adverse Event Definitions

Term	Definitions per FDA Final Rule
Adverse Event	Any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related
Suspected adverse reaction	Any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, "reasonable possibility" means there is evidence to suggest a causal relationship between the drug and the adverse event. Suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.
Unexpected adverse event or unexpected suspected adverse reaction	An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed. Includes adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

Term	Definitions per FDA Final Rule
Serious adverse event or serious suspected adverse reaction	Any untoward medical occurrence that at any dose, in the opinion of the investigator or sponsor: • Results in death, Is life-threatening, NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe. • Results in a persistent or significant incapacity or a substantial disruption of the ability to conduct normal life functions, • Is a congenital anomaly/birth defect. • Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered serious when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

11.2. Eliciting Adverse Event Reports

Simple, unbiased questions should be asked to elicit reports of AEs during the clinical study. For example:

- How have you felt since your last visit?
- Have you had any health problems since you were last here?

11.3. Assessment and Recording of Adverse Events

All AEs grade 3 or higher occurring during the reporting period must be assessed and recorded as follows:

- Name of the event (diagnosis preferred, otherwise state signs and symptoms)
- Date of onset and stop date (if available)
- Course (i.e., intermittent, ongoing)
- Maximum intensity or severity (grade) based on the NCI CTCAE version 4.03
- Relationship to study treatment
- Seriousness
- Outcome
- Use of concomitant medication to treat a patient for an AE

Adverse events of special interest that occur at grade 1 or higher will be recorded. Adverse events of special interest are: mucositis, infection, neutropenia, diarrhea, and thrombocytopenia.

11.4. Guidelines for Recording Adverse Events

Each reportable AE should be documented as a single medical diagnosis. If this is not possible, the signs or symptoms should be documented until a medical diagnosis is identified. If an AE resolves and reoccurs at a later date, a new AE must be documented and reported as appropriate. The following guidelines should be followed to improve the quality and precision of AE data:

- Recognized medical terms (not colloquialisms, abbreviations, or jargon) should be used when recording AEs
- The diagnosis (i.e., disease or syndrome) should be recorded instead of signs and symptoms (e.g., pneumonia instead of shortness of breath, coughing, and fever)
- Any sign or symptom considered as unrelated to an encountered disease or syndrome should be recorded as an individual AE (e.g., if nausea and severe headache are observed at the same time, each event should be recorded as a separate AE)
- Adverse events occurring within 30 days after EOT should be reported.

11.5. Intensity of the Adverse Event

The investigator will grade each reportable AE based on its maximum intensity (or severity) using the NCI CTCAE V4.03 (available at: http://evs.nci.nih.gov/ftp1/CTCAE/About.html). [31] If no grading criteria are provided for the AE, it should be graded using the scale presented in Table 5, noting that not all grades are appropriate for all AEs.

11.6. Adverse Event Attribution

The investigator will determine the attribution of toxicity relative to the vosaroxin, cytarabine, other concomitant medication, or other medical causes in accordance with the ICH E2A guidelines.

Table 5. Definition of Adverse Event Attribution

Attribution	Description
Unrelated	The AE is *clearly NOT* related to the intervention.
Unlikely:	The AE is *doubtfully related* to the intervention.
Possible:	The AE *may be related* to the intervention.
Probable:	The AE is *likely related* to the intervention.
Definite:	The AE is *clearly related* to the intervention.

11.7. Serious Adverse Event Reporting Requirements

Study site personnel must report all SAEs by fax as soon as possible to the Coordinating Center, but within 1 business day of first becoming aware of the SAE. The following minimum information is required by the sponsor:

- Patient identification (i.e., study number, sex, age)
- Description of the SAE (diagnosis preferred, symptoms, etc.)
- Investigator name
- Study drug and causal relationship of the SAE to the study drug(s)

The SAE report should be submitted via secure email transfer or via fax using the contact information listed below:

Attn: VICC Multi-Institutional Coordinating Center
Fax: 615-875-0040
or
Coordinating.Center@vanderbilt.edu

Transmission of the SAE report should be confirmed by the site personnel submitting the report.

Follow-up information for SAEs and information on non-serious AEs that become serious should also be reported to the Coordinating Center as soon as it is available; these reports should be submitted using the SAE Report Form.

Investigators must submit written safety reports as required by their Institutional Review Board (IRB) or Institutional Ethics Committee (IEC) within timelines set by regional regulations (European Union common technical document or US FDA). The study site should retain documentation of the submission of expedited safety reports to the IRB/IEC, and their receipt.

Persistent or recurrent AML (or "disease progression"), including AML leading to death, should not be recorded as an SAE. Persistent or recurrent AML is an outcome of treatment and will be documented as treatment failure. Associated events resulting from persistent or recurrent AML (or "disease progression") should be recorded as AE or SAEs, and SAEs resulting in death should be documented.

11.8. General Instructions for Reporting Serious Adverse Events to the FDA and Sunesis Pharmaceuticals

Food and Drug Administration (FDA)

FDA regulations and statutory provisions establish adverse event reporting requirements for human drugs. These requirements apply to the manufacturer of the product in question. In general, manufacturers of prescription drugs marketed for human use are required to submit postmarketing safety reports of adverse drug experiences to FDA.[a] A report of each adverse drug experience that is both serious and unexpected must be made to FDA as soon as possible, but no later than 7 days for a life-threatening or fatal event, and 15 days for all others after receiving information about the event.[b] Persons required to file such 15-day Alert reports are also required to investigate and submit any new information to FDA.[c] The Sponsor is responsible for reporting SAEs to the FDA.

SAE supporting documents, such as a discharge summary or pertinent laboratory data may be provided; however, the study personnel should not wait to receive complete information before notifying the Coordinating Center of an SAE. The study site is responsible for requesting pertinent follow-up information missing from initial reports and forwarding the information within 24 hours of receipt. The Coordinating Center may request additional information if necessary.

[a] 21 C.F.R. §§ 310.305(c); 314.80(c); 314.98 (human drugs), 600.80(c) (biologics). To avoid duplication of reports, non-applicant manufacturers, packers, and distributors of drug and biological products having an approved application may submit all reports of serious adverse drug experiences to the applicant within 5 calendar days of receipt of information about the event, rather than submitting reports to FDA. See 21 C.F.R.

§§ 314.80(c)(1)(iii), 600.80(c)(1)(iii). Similarly, packers and distributors of prescription drug products marketed for human use without an approved application may meet their post-marketing 15-day safety reporting obligations under 21 C.F.R. § 310.305 by submitting all reports of serious adverse drug experiences to the manufacturer within 5 calendar days of the receipt of information instead of reporting to FDA. Applicants/manufacturers receiving such data must then, in turn, submit a 15-day Alert report to FDA.

[b] 21 C.F.R. §§ 310.305(c)(1); 314.80(c)(1)(i); 314.98(a) (human drugs), 600.80(c)(1)(i) (biologics). There are also certain specific reporting requirements concerning fatalities related to blood and blood products. 21 CFR §§ 606.170(b), 640.73.

[c] 21 C.F.R. §§ 310.305(c)(2), 314.80(c)(1)(ii), 314.98(a) (human drugs), 600.80(c)(1)(ii) (biologics).

Sunesis Pharmaceuticals

The Sponsor will report all SAEs regardless of causal relationship to study treatment to Sunesis.

12. STATISTICAL METHODS AND DETERMINATION OF SAMPLE SIZE

12.1. Statistical Considerations

The primary objective of this single-arm, phase II clinical trial is to determine if Vosaroxin, in combination with standard dose cytarabine as induction chemotherapy, can significantly improve the rate of complete remission (CR) in patients with untreated acute myelocytic leukemia. Forty-one (41) patients will be enrolled in a two-stage design that permits one interim look to examine evidence of futility. As per convention, the study will not be stopped early on the basis of preliminary evidence of efficacy observed at the end of the first stage (i.e., at the interim look).

12.2. Sample Size Considerations

In accordance with our desire to maintain a uniform study design, we set a total sample size of 41 participants. An interim futility analysis will be conducted following enrollment of 17 evaluable patients. If ≤ 7 patients achieve CR then the likelihood ratio in support of reaching the goal endpoint of $\geq 60\%$ remission would be diminished and the data would then be reviewed by a DSMB to determine if further enrollment is warranted. If > 7 CRs are experienced by the first 17 evaluable patients, enrollment will automatically proceed to enroll a total of 41 patients. Under this two-stage design, a 60% complete remission rate by the "7+V" combination is at least 3.375 times more likely than a 40% CR rate if 22 or more patients experience CR.

In the context of the planned likelihood assessment the option will be provided to add an extension cohort of up to 20 participants if the initial trial results are inconclusive. The use of a likelihood based extension cohort results in false positive and false discovery rates of 3.7% and 3.9%, respectively.

12.3. Study Design

Overview: Two-stage single-arm phase II designs with a single interim look for futility are common in this setting. Simon's optimal two-stage design[32] is a classic design and remains a popular choice. Herein we propose, in the context of a standard two-stage design, that a likelihood-based assessment of the statistical evidence be used The unique and informative assessment of the observed evidence provided by likelihood ratios is reason enough to employ the approach.

We will employ the likelihood ratio (LR), not a tail area probability (p-value), to represent the strength of statistical evidence with respect to either a 40% or 60% true CR rate. Likelihood ratios are positive and a LR of 1 indicates completely neutral evidence. Small likelihood ratios ($LR \ll 1$) indicate evidence for the null hypothesis and large likelihood ratios ($LR \gg 1$) indicate evidence for the alternative. The scale of evidence is truly continuous, mapping a gradual shift from weak to moderate to strong evidence.

An important advantage is that, unlike p-values, likelihood ratios have three evidential zones: evidence favoring the null hypothesis, evidence favoring the alternative hypothesis, and weak or inconclusive evidence (i.e., favoring neither hypothesis). Of course, p-values have only two zones: evidence against the null hypothesis ($p<0.05$) and inconclusive evidence ($p>0.05$; large p-values do not constitute stronger evidence in favor of the null hypothesis). Moreover, the likelihood false discovery rate is easily quantifiable as $1/(LR+1)$. Thus, an observed likelihood ratio of 4 (supporting the alternative) has a false discovery rate of 20%. Lastly, likelihood designs have excellent operating characteristics.

Parameters and assumptions: For planning purposes, we assume the complete remission rate of standard induction chemotherapy is 40% in the targeted population of this trial and that the use of Vosaroxin in combination with cytarabine may increase this rate to 60%. We plan to enroll a total of 41 patients, but provide the option of adding an extension cohort of 10 to 20 participants if the initial trial results are inconclusive.

Two-stage single-arm phase II designs: There are many two-stage designs that meet nominal Type I and II Error criteria. We choose a two-stage design with a slightly increased expected sample size (EN) of 25.6 but keeps the two-stage sample size below that of a single-stage study (n=42) as follows: In stage I, 17 patients are accrued and the study is stopped only if 7 or fewer patients experience a complete remission (CR). Stopping in this fashion is interpreted as evidence of futility. If 8 or more patients experience a CR, the study continues to accrue an additional 24 participants (for a total of 41). At the end, the study is said to generate evidence in support of the hypothesis that incorporating Vosaroxin into frontline AML induction chemotherapy is worthy of future study if 22 or more patients experience CR (i.e. if the observed response rate is greater than 53%). If frontline induction therapy with Vosaroxin plus standard dose continuous infusion cytarabine fails to improve upon the historical CR rate of 40% with conventional 7+3 induction therapy, the study has a 64% probability of early termination (PET).

A standard (frequentist) two-stage design is unable to handle deviations from its planned design (i.e., when the sample size is anything other than 17 or 41). This occasionally happens in multi-center clinical trials for practical reasons relating to the speed of communication and clinical expediency. Green and Dahlberg[33] introduced flexible designs to that are able to accommodate such deviations from planed designs with minimal impact to their nominal operating characteristics. However, in this case, the Green and Dahlberg criteria result in a 25% PET, which poorly protects patients from ineffective therapy. This is one motivating factor in our choice of employing a likelihood-based assessment of the evidence. The likelihood ratio reveals alternative interim stopping criteria that maintain nominal Type I and Type II Error rates, adding significant logistic flexibility (Table 6). Within the context of standard two-stage design, we believe our proposed design best balances the probability of early termination and expected sample size under the stated constraints.

Table 6. Interim stopping rules that are constrained to lie between 30% and 60% of total accrual and have a probability of early termination that exceeds 50%. Type I & II Error rates are below designed nominal levels (5% & 20%).

Observed CRs in stage I	Stage I sample size	Probability of early termination	Expected sample size	Likelihood ratio favoring null that corresponds to Simon's futility stopping rule
7	17	64%	25.6	1/3.375
8	19	67%	26.3	1/3.375
9	21	69%	27.2	1/3.375
10	23	71%	28.2	1/3.375
6	16	53%	27.8	1/5.062
7	18	56%	28.2	1/5.062
8	20	60%	28.5	1/5.062

Likelihood assessment of evidence: In accordance with our desire to maintain a uniform study design, we set a total sample size of 41 participants, a 17 patient first stage, and an interim futility criterion of 7 or fewer CRs with the added flexibility at the interim analysis indicated in table 1. The interim futility criterion translates to stopping the study at the end of the first stage if the observed likelihood ratio is less than 1/3.375 (i.e., if the evidence better supports the null hypothesis by a factor of at least 3.375). The likelihood ratio is defined as $LR = {\theta_1}^r(1-\theta_1)^{n-r}/{\theta_0}^r(1-\theta_0)^{n-r}$ where r is the number of CR's among n patients treated and $H_0{:}\,\theta = \theta_0 = 0.4$, $H_1{:}\,\theta = \theta_1 = 0.6$. The translated stopping LRs for the competing designs are listed in the last column of table 1.

An important aspect of the likelihood assessment is that it can quantify the reliability of an observed outcome. For example, while the study would stop early with either an observed LR of 1/3.375 or 1/15 (evidence favoring the null hypothesis), the associated false discovery rates of 0.23 (=1/4.375) and 0.063 (=1/16), respectively, distinguish between those results and provide critical information for investigators when interpreting this evidence at the end of the study.

With a likelihood design, it can happen that the trial yields weak or inconclusive evidence. Technically this can happen in all standard (i.e., frequentist) two-stage designs as well, but when it does the standard design forces a 'call' of either 'Reject the null' or 'Fail to reject the null' (the assignment depends on how the Type I Error is spent throughout the study and can lead to some uncomfortable circumstances, such as calling evidence supporting the alternative as 'Fail to reject' and vice versa). Hence, in likelihood designs, it is desirable to minimize the chance of observing weak evidence. Our proposed two-stage design accomplished this sufficiently, as we next describe, but the important thing to remember is that if the trial does result in weak evidence there is no penalty for accruing additional subjects and reporting the combined evidence (assuming that resources will allow such action). In a likelihood design, the probability of weak evidence is driven to zero by the sample size, so it is always possible to refine inconclusive results, often within limited resource constraints.

Table 7 displays a simulation of the proposed trial, potential outcomes, and their probability of occurrence. Analytic expressions for these probabilities exist, but simulating is simpler and essentially as accurate. Table 2 shows what we expect to happen, on average, for our proposed design and what happens if the sponsor chooses to add patients at the end of the trial to refine weak evidence (if observed). In particular, it shows that the chance of observing weak evidence at the end of the study is ~8%, which can be reduce to ~3% (or ~1%) by adding 10 (or 20) more participants. Importantly, only an additional 54 false positives were observed, which represents a very modest inflation in the Type I Error rate from 4.9% (=247/5000) to 6%, and 286 trials under the alternative achieved sufficient evidence to declare efficacy for an increase in power from 80% (=4026/5000) to 86%. At the end of stage II, the false discovery rate for any type of rejection (i.e., any likelihood ratio greater than or equal to 3.375) is 5.8% (=247/(247+4026)). Clearly, cohort expansion can proceed with minimal rates of unwanted consequences.

Table 7. Simulation results for extending the likelihood two stage trial for the purpose of refining weak evidence. Cells contain the number (proportion of 5000) trials in each evidentiary class.

Results Under True Ho:						
LR Class	Trials at end of two-stage design	Weak evidence	Add 10 patients	Weak evidence	Add 10 patients	Weak evidence
LR ≤ 1/3.375	1145		207		105	
1/3.375<LR<1	**260**	424 (8.5%)	**113**	179 (3.6%)	**35**	58 (1.2%)
1<LR<3.375	**164**		**66**		**23**	
LR ≥ 3.375	247		38		16	
Results under true Ha:						
LR ≤ 1/3.375	143		39		16	
1/3.375<LR<1	**148**	397 (7.9%)	**48**	135 (2.7%)	**30**	134 (1.1%)
1<LR<3.375	**249**		**87**		**26**	
LR ≥ 3.375	4026		223		63	

[a] 64% (3184) and 8.7% (434) trials stopped early for futility under the true null and true alternative hypotheses, respectively.

Under the likelihood evidentiary paradigm observed results are reported as follows (let K>1 be the pre-specified LR that maintains a certain false discovery rate): 1) sufficiently strong evidence for the null (LR ≤ 1/K) ; 2) actionable weak evidence for the null (1/K<LR<1) ; 3) actionable weak evidence for the alternative (1<LR<K) ; 4) sufficiently strong evidence for the alternative (LR ≥ 1/K). We will also report the FDR for the result at hand, i.e. 1/(LR+1). As detailed below, this trial may recruit additional participants if the observed LR is weak (i.e., 1/K<LR<K) with a maximum of 61 participants.

A significant number (~8%) of discoveries under the null and alternate hypotheses for our two-stage design had LR of exactly 3.375. Though such results would allow the conclusion of sufficient activity to warrant further investigation in more definitive trials because it passes nominal Type I and II Error rate criteria, we propose to further evaluate these results in the extension cohorts by setting K=4, treating those trials with LR's of 3.375 as weak evidence. The PET remains unaffected by this slight change. However, the design that uses K=4 and a total sample size of 61 patients has a false positive rate that decreases to 3.7% and power that increases to 83.7%. This strategy also decreases the false discovery rate to 3.9% (table 8). Hence such a design is quite feasible.

Table 8. Simulation results for extending the likelihood two stage trial for the purpose of refining weak evidence for K=4. Cells contain the number (proportion of 5000) trials in each evidentiary class.

Results Under True Ho:						
LR Class	Trials at end of two-stage design	Weak evidence	Add 10 patients	Weak evidence	Add 10 patients	Weak evidence
LR ≤ 1/4	878		307		186	
1/4<LR<1	**527**	813 (16.3%)	**299**	474 (9.5%)	**175**	273 (5.4%)
1<LR<4	**286**		**175**		**98**	
LR ≥ 4	125		32		15	
Results under true Ha:						
LR ≤ 1/4	65		20		21	
1/4<LR<1	**226**	833 (16.7%)	**170**	471 (9.4%)	**85**	274 (5.5%)
1<LR<4	**607**		**301**		**189**	
LR ≥ 4	3668		342		176	

[a] 64% (3184) and 8.7% (434) trials stopped early for futility under the true null and true alternative hypotheses, respectively.

General Statistical Considerations and Analysis Plan

Categorical variables (e.g. gender, response) will be summarized in frequency tables using counts and percentage of patients by group. Continuous variables (e.g. age) will be summarized using the minimum and maximum values, quartiles, mean and standard deviation. 95% confidence intervals will be estimated for all variables. All comparisons will be two-sided and statistical significance is declared for p-values < 0.05. All patients receiving at least one dose of therapy will be included in efficacy and safety analyses.

12.4. Definition of Endpoints Response for Interim Analysis.

Response for interim analysis will be defined as the best response prior to or on the day-84 bone marrow biopsy.

- Leukemia-free Survival (LFS or DFS) is defined as the time from complete remission to disease progression or death for any reason. Patients who are disease free and alive as of last follow-up will be censored at their last follow-up date.
- Event-free survival is defined as the time from start of therapy to progression or death for any reason. Patient progression-free and alive at last follow-up will be censored at their last follow-up date.
- Overall survival is defined at the time from start of therapy to death for any reason. Patients alive as of last follow-up will be censored at their last follow-up date.

12.5. Statistical Comparisons

Categorical variables will be compared among important patient subgroups using the chi-square test or Fisher's exact test, as appropriate. Continuous variables will be compared using rank based non-parametric tests. Survival distributions will be estimated using the method of Kaplan and Meier and comparing these distributions among patient subgroups will be made using the logrank test. Multivariable models of response and survival estimates will be constructed using logistic and Cox (proportional hazards) regression. Time to response will be implemented as a time-dependent covariate in models assessing LFS.

12.6. Safety Analysis

Adverse events and laboratory data will be graded according to the NCI CTCAE version 4.03. As described above frequency and percentage of patients will be summarized for any adverse event by grade, attribution, organ class, and preferred term overall and by patient. Change in continuous laboratory data from just prior to therapy and by visit will be summarized by frequency of abnormal values. Linear and non-linear regression will be used to model changes over time, accounting for intra-patient correlation using mixed models or GEE, as appropriate.

13. INVESTIGATOR AND ADMINISTRATIVE REQUIREMENTS

13.1. Compliance With Laws and Regulations

The investigator will conduct this clinical study in compliance with the protocol; Title 21 of the Code of Federal Regulations (CFR),[34] current Good Clinical Practice (GCP); ICH guidelines, including E6-GCP Consolidated Guidance;[35] the Declaration of Helsinki;[36] the requirements of other regulatory agencies as necessary; and local ethical and legal requirements.

To the extent applicable, the investigator will interpret all references to the FDA; Federal Food, Drug, and Cosmetic Act; CFR; ICH; GCP; and the like; as also referring to any corresponding requirements of local regulatory agencies, regulations, and laws. If there is any discrepancy between local, FDA, and ICH requirements, the most stringent standard will apply.

Vanderbilt is the Sponsor of this study. All aspects of the study will be carefully monitored by the Sponsor for compliance with applicable government regulations with respect to current GCP and standard operating procedures.

13.1.1. Ethics

Ethical Conduct of the Study and Independent Ethics Committees

As required by FDA regulation (21 CFR 56) and ICH guidelines for GCP, the Investigator must obtain Institutional Review Board (IRB) or Institutional Ethics Committee (IEC) review and approval of the study protocol, Informed Consent Forms

(ICFs), patient recruitment materials, and any other pertinent documents before any study-related activities involving patients are performed.

As required in 21 CFR 50, the investigator or designee must comply with the informed consent process, and ensure that each patient enrolled in this clinical study understands the information presented in the IEC-approved ICF and agrees voluntarily to participate in the clinical study.

The investigator must inform the IEC of the progress of the clinical study and report any non-administrative changes made to the protocol; in any case, the investigator must provide an update to the IEC at least once yearly or per country-specific guidelines.

13.1.2. IRB Approval

The trial protocol, ICF, IB, available safety information, any patient documents, patient recruitment procedures (e.g., advertisements), information about payments (i.e., PI payments) and compensation available to the patients should be submitted to the IRB for ethical review and approval if required by local regulations, prior to the trial start.

Information regarding study conduct and progress will be reported to the Institutional Review Board (IRB) per the current institutional standards of each participating institution.

The Study Chair (or his designee) is responsible for the coordination and development of all protocol amendments. Once approved by the Chair, Vanderbilt will disseminate this information to participating institutions.

IRB approval for protocol amendments and changes to the informed consent document must be obtained per the current institutional standards at each participating institution.

Any change to the protocol and informed consent document must be reviewed and approved by the Coordinating Center before being submitted to the Institutional Review Board/Independent Ethics Committee at participating institutions. Amendments should not be implemented until all necessary approvals have been obtained, except when necessary to eliminate an immediate hazard to study subjects.

Safety updates for will be prepared by the Coordinating Center as required, for submission to the relevant IRB.

13.1.3. Informed Consent

Informed consent is a process by which a subject voluntarily confirms his or her willingness to participate in a particular trial, after having been informed of all aspects of the trial that are relevant to the subject's decision to participate. Informed consent is documented by means of a written, signed and dated informed consent form.

The informed consent form will be submitted for approval to the IRB that is responsible for review and approval of the trial. Each consent form must include all of the relevant elements currently required by the FDA, as well as local county authority or state regulations and national requirements.

Before recruitment and enrollment into the trial, each prospective candidate will be given a full explanation of the trial. Once the essential information has been provided to the prospective candidate, and the investigator is sure that the individual candidate understands the implications of participating in this trial, the candidate will be asked to give consent to participate in the trial by signing an informed consent form. A notation that written informed consent has been obtained will be made in the patient's medical record. A copy of the informed consent form, to include the patient's signature, will be provided by the investigator to the patient.

If an amendment to the protocol substantially alters the trial design or the potential risks to the patients, the patient's consent to continue participation in the trial must be obtained.

Any change to the informed consent document must be reviewed and approved by the Coordinating Center before being submitted to the Institutional Review Board/Independent Ethics Committee at participating institutions.

13.1.4. Data Collection

The trial CRF is the primary data collection instrument for the trial. CRFs will be completed using the English language and should be kept current to enable the monitor to review the patients' status throughout the course of the trial.

In order to maintain confidentiality, only trial number, patient number, initials and date of birth will identify the patient in the CRF. If the patient's name appears on any other document (e.g. laboratory report), it must be obliterated on the copy of the document to be supplied to Coordinating Center and replaced instead with the patient number and patient's initials. The investigator will maintain a personal patient identification list (patient numbers with corresponding patient identifiers) to enable records to be identified and verified as authentic. Patient data/information will be kept confidential, and will be managed according to applicable local, state, and federal regulations.

All data requested on the CRF must be supported by and be consistent with the patient's source documentation. All missing data must be explained. When a required laboratory test, assessment, or evaluation has not been done or an "Unknown" box is not an option on the CRF, a note should be created verifying that the field was "Not Done" or "Unknown". For any entry errors made, the error(s) must be corrected, and a note explaining the reason for change should be provided.

The investigator will electronically sign and date the patient CRF casebook indicating that the data in the CRF has been assessed. Each completed CRF will be signed and dated by the PI, once all data for that patient are final.

13.1.5. Trial Monitoring

Vital Study Data and Safety Monitoring Board (DSMB)

The VITAL Study DSMB (consisting of a representative from the funding organization, the study chairs, a biostatistician, and participating investigator to be selected by the study chairs) will review the clinical data to determine the merits of continued enrollment.

VICC Data and Safety Monitoring Committee (DSMC)

Quarterly safety and monitoring reports are available to the DSMC as determined by the VICC Scientific Review Committee (SRC). The DSMC reviews all adverse events reported during the previous month for all clinical trials active at the VICC and makes recommendations to address concerns of patient safety. The DSMC of the VICC SRC will submit an annual report to the VICC Director on activities of the preceding year and will make recommendations to improve data and safety monitoring activities as needed.

A Quality Assurance auditor under the direction of the VICC DSMC will audit this clinical trial quarterly for compliance with adverse event reporting, regulatory and studies requirements, and data accuracy and completion. Audit reports detailing the findings are provided to the DSMC.

Investigators will allow auditors access to all pertinent medical records, as required by federal regulations, in order to allow for the verification of data gathered in the data case report forms (CRFs) and for the review of the data collection process. At visits, the auditor may review various aspects of the trial including, but not limited to: screening and enrollment logs; compliance with the protocol and with the principles of Good Clinical Practice; completion of case report forms; source data verification; study drug accountability and storage; facilities and staff; and regulatory compliance.

VICC Multi-Institutional Coordinating Center

The trial additionally will be monitored by the VICC Multi-Institutional Coordinating Center. The actual frequency of monitoring will depend on the enrollment rate and performance of the site. Monitoring will be conducted through onsite and remote monitoring, teleconferences with the Investigator and site staff, and appropriate communications by mail, fax, email, or telephone. The purpose of

monitoring is to ensure that the study is conducted in compliance with the protocol, standard operating procedures (SOPs), and other written instructions, and to ensure the quality and integrity of the data.

During scheduled monitoring visits, investigators and the investigational site staff must be available to discuss the progress of the trial, make necessary corrections to case report form entries, respond to data clarification requests, provide required regulatory documents, and respond to any other trial-related inquiries of the monitor.

In addition to the above, the FDA may review the conduct or results of the study at the investigational site.

13.1.6. Closure of the Study

The sponsor reserves the right to discontinue a site at any time during the study for medical or administrative reasons such as:

- Unsatisfactory enrollment;
- GCP noncompliance;
- Inaccurate or incomplete data collection;
- Falsification of records;
- Failure to adhere to the study protocol.

13.1.7. Records Retention

U.S. FDA regulations (21 CFR §312.62[c]) require that records and documents pertaining to the conduct of this study and the distribution of investigational drug, including CRFs, consent forms, laboratory test results, and medication inventory records, must be retained by each Principal Investigator for two years after marketing application approval. If no application is filed, these records must be kept two years after the study is discontinued and the U.S. FDA and the applicable national and local health authorities are notified.

Following closure of the study, each participating center will maintain a copy of all site study records in a safe and secure location. The Sponsor will inform the Investigator at each site at such time that the records may be destroyed.

References

1. Siegel R, Ma J, Zou Z, Jemal A. Cancer statistics, 2014. *CA: a cancer journal for clinicians* 2014; **64**(1): 9-29.
2. Yates JW, Wallace HJ, Jr., Ellison RR, Holland JF. Cytosine arabinoside (NSC-63878) and daunorubicin (NSC-83142) therapy in acute nonlymphocytic leukemia. *Cancer Chemother Rep* 1973; **57**(4): 485-8.
3. Bloomfield CD, Goldman A, Hassfeld D, de la Chapelle A. Fourth International Workshop on Chromosomes in Leukemia 1982: Clinical significance of chromosomal abnormalities in acute nonlymphoblastic leukemia. *Cancer Genet Cytogenet* 1984; **11**(3): 332-50.
4. Breems DA, Van Putten WL, De Greef GE, et al. Monosomal karyotype in acute myeloid leukemia: a better indicator of poor prognosis than a complex karyotype. *J Clin Oncol* 2008; **26**(29): 4791-7.
5. Grimwade D, Hills RK, Moorman AV, et al. Refinement of cytogenetic classification in acute myeloid leukemia: determination of prognostic significance of rare recurring chromosomal abnormalities among 5876 younger adult patients treated in the United Kingdom Medical Research Council trials. *Blood* 2010; **116**(3): 354-65.
6. Grimwade D, Walker H, Oliver F, et al. The importance of diagnostic cytogenetics on outcome in AML: analysis of 1,612 patients entered into the MRC AML 10 trial. The Medical Research Council Adult and Children's Leukaemia Working Parties. *Blood* 1998; **92**(7): 2322-33.
7. Rollig C, Bornhauser M, Thiede C, et al. Long-term prognosis of acute myeloid leukemia according to the new genetic risk classification of the European LeukemiaNet recommendations: evaluation of the proposed reporting system. *J Clin Oncol* 2011; **29**(20): 2758-65.
8. SEER Cancer Statistics Review 1975-2010. *National Cancer Institute*.
9. Farag SS, Archer KJ, Mrozek K, et al. Pretreatment cytogenetics add to other prognostic factors predicting complete remission and long-term outcome in patients 60 years of age or older with acute myeloid leukemia: results from Cancer and Leukemia Group B 8461. *Blood* 2006; **108**(1): 63-73.
10. 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): 1312-20.
11. Stuart RK CL, Maris MB, et al. Voreloxin single agent treatment of older patients >/= 60 years with previously untreated acute myeloid leukemia: results from phase 2 study with 3 schedules. . *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2010; **28**(15s): suppl; abstr 6525.
12. Lancet JE, Ravandi F, Ricklis RM, et al. A phase Ib study of vosaroxin, an anticancer quinolone derivative, in patients with relapsed or refractory acute leukemia. *Leukemia* 2011; **25**(12): 1808-14.
13. Roboz GJ LJ, Cripe LD et al. Final results of a Phase II pharmacokinetic/pharmacodynamic (PK/PD) study of combination voreloxin and cytarabine in patients with relapsed or refractory acute myeloid leukemia. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2010; (15s): (suppl; abstr 6526).
14. Ravandi F, Ritchie E, Sayar H, et al. Improved Survival in Patients with First Relapsed or Refractory Acute Myeloid Leukemia (AML) Treated with Vosaroxin Plus Cytarabine Versus Placebo Plus Cytarabine: Results of a Phase 3 Double-Blind Randomized Controlled Multinational Study (VALOR); 2014.
15. Elsea SH, Westergaard M, Burden DA, Lomenick JP, Osheroff N. Quinolones share a common interaction domain on topoisomerase II with other DNA cleavage-enhancing antineoplastic drugs. *Biochemistry* 1997; **36**(10): 2919-24.

16. Tomita K, Tsuzuki Y, Shibamori K, et al. Synthesis and structure-activity relationships of novel 7-substituted 1,4-dihydro-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylic acids as antitumor agents. Part 1. *Journal of medicinal chemistry* 2002; **45**(25): 5564-75.
17. Tsuzuki Y, Tomita K, Shibamori K, Sato Y, Kashimoto S, Chiba K. Synthesis and structure-activity relationships of novel 7-substituted 1,4-dihydro-4-oxo-1-(2-thiazolyl)-1,8-naphthyridine-3-carboxylic acids as antitumor agents. Part 2. *Journal of medicinal chemistry* 2004; **47**(8): 2097-109.
18. 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.
19. Wong OK, Conroy A, Tan N, et al. 598 POSTER Voreloxin (formerly SNS-595) is a potent DNA intercalator and topoisomerase II poison that induces cell cycle dependent DNA damage and rapid apoptosis in cancer cell lines. *European Journal of Cancer Supplements* 2008; **6**(12): 187.
20. 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): 393-9.
21. Advani RH, Hurwitz HI, Gordon MS, et al. Voreloxin, a first-in-class anticancer quinolone derivative, in relapsed/refractory solid tumors: a report on two dosing schedules. *Clinical cancer research : an official journal of the American Association for Cancer Research* 2010; **16**(7): 2167-75.
22. Lancet JE, Roboz GJ, Cripe LD, et al. A phase 1b/2 study of combination vosaroxin and cytarabine in patients with relapsed or refractory acute myeloid leukemia. *Haematologica* 2014.
23. 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. *British journal of haematology* 2014.
24. Stuart RK, Ravandi Kashani F, Cripe LD, et al. Voreloxin single-agent treatment of older patients (60 years or older) with previously untreated acute myeloid leukemia: Final results from a phase II study with three schedules. *ASCO Meeting Abstracts* 2010; **28**(15_suppl): 6525.
25. Daver NG, Kantarjian HM, Pierce S, et al. Phase I/II study of vosaroxin and decitabine in older patients (pts) with acute myeloid leukemia (AML) and high-risk myelodysplastic syndrome (MDS). *ASCO Meeting Abstracts* 2014; **32**(15_suppl): 7098.
26. Sorror ML, Maris MB, Storb R, et al. Hematopoietic cell transplantation (HCT)-specific comorbidity index: a new tool for risk assessment before allogeneic HCT. *Blood* 2005; **106**(8): 2912-9.
27. Wheatley K, Brookes CL, Howman AJ, et al. Prognostic factor analysis of the survival of elderly patients with AML in the MRC AML11 and LRF AML14 trials. *Br J Haematol* 2009; **145**(5): 598-605.
28. Krug U, Rollig C, Koschmieder A, et al. Complete remission and early death after intensive chemotherapy in patients aged 60 years or older with acute myeloid leukaemia: a web-based application for prediction of outcomes. *Lancet* 2010; **376**(9757): 2000-8.
29. Mosteller RD. Simplified calculation of body-surface area. *The New England journal of medicine* 1987; **317**(17): 1098.
30. 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. *Journal of clinical oncology : official journal of the American Society of Clinical Oncology* 2003; **21**(24): 4642-9.
31. Common Terminology Criteria for Adverse Events (CTCAE) version 4.03. National Cancer Institute, National Institutes of Health, U.S. Department of Health and Human Services. June 14, 2010.
32. Simon R. Optimal two-stage designs for phase II clinical trials. *Controlled clinical trials* 1989; **10**(1): 1-10.
33. Green SJ, Dahlberg S. Planned versus attained design in phase II clinical trials. *Statistics in medicine* 1992; **11**(7): 853-62.
34. Code of Federal Regulations. Title 21, Parts 170 to 199; Revised April 1, 2012.
35. Guidance for Industry. E6 Good Clinical Practice: Consolidated Guidance ed. ICH; April 1996.

36. World Medical A. World Medical Association Declaration of Helsinki. Ethical principles for medical research involving human subjects. *Bulletin of the World Health Organization* 2001; **79**(4): 373-4.
37. Oken MM, Creech RH, Tormey DC, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group. *American journal of clinical oncology* 1982; **5**(6): 649-55.
38. Freifeld AG, Bow EJ, Sepkowitz KA, et al. Clinical practice guideline for the use of antimicrobial agents in neutropenic patients with cancer: 2010 Update by the Infectious Diseases Society of America. *Clin Infect Dis* 2011; **52**(4): 427-31.

Appendix A. Eastern Cooperative Oncology Group Performance status[37]

GRADE	ECOG PERFORMANCE STATUS
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities; up and about more than 50% of waking hours
3	Capable of only limited selfcare; confined to bed or chair more than 50% of waking hours
4	Completely disabled; cannot carry on any selfcare; totally confined to bed or chair
5	Dead

*Oken M, Creech R, Tormey D, et al. Toxicity and response criteria of the Eastern Cooperative Oncology Group.*Am J Clin Oncol.* 1982;5:649-655.

Appendix B. WHO diagnostic criteria for AML

The diagnosis of AML required a marrow or blood blast count of 20% or more, except for AML with t(15;17), t(8;21), inv(16) or t(16;16), and some cases of erythroleukemia. Myeloblasts, monoblasts, and megakaryoblasts are included in the blast count. Monoblasts and promonocytes, but not abnormal monocytes, are counted as blast equivalents in AML with monocytic or myelomonocytic differentiation. Erythroblasts are not counted as blasts except in the rare instance of pure erythroid leukemia.

Categories:

AML with recurrent cytogenetic abnormalities
- AML with t(8;21)(q22;q22); RUNX1-RUNX1T1
- AML with inv(16)(p13.1q22) or t(16;16)(p13.1;q22);
- Acute promyelocytic leukemia with t(15;17)(q22;q12);
- AML with t(9 ;11)(p22;q23);
- AML with t(6;9)(p23;q34);
- AML with inv(3)(q21q26.2) or t(3;3)(q21;q26.2)
- AML with mutated NPM1
- AML with mutated CEBPA

AML with myelodysplasia-related changes

Complex Karyotype (≥3 abnormalities)

Unbalanced abnormalities
- -7/del(7q)
- -5/del(5q)
- i(17q)/t(17p)
- -13/del(13q)
- del(11q)
- del(12p)/t(12p)
- del(9q)
- idic(X)(q13)

Balanced abnormalities
- t(11;16)(q23;q13.3)
- t(3;21)(q26.2;q22.1)
- t(1;3)(p36.3;q21.1)
- t(2;11)(p21;q23)
- t(5;12)(q33;p12)
- t(5;7)(q33;q11.2)
- t(5;17)(q33;p13)

t(5;10)(q33;q21)
t(3;5)(q25;q34)

Therapy-related myeloid neoplasms
- Myeloid Sarcoma
- AML, Not Otherwise Specified
- AML with minimal differentiation
- AML without maturation
- AML with maturation
- Acute myelomonocytic leukemia
- Acute monoblastic and monocytic leukemia
- Acute erythroid leukemia
- Acute megakaryoblastic leukemia
- Acute basophilic leukemia
- Acute panmyelosis with myelofibrosis

Appendix C. AML Risk prognostication

National Comprehensive Cancer Network®

NCCN Guidelines Version 1.2015
Acute Myeloid Leukemia

NCCN Guidelines Index
AML Table of Contents
Discussion

RISK STATUS BASED ON VALIDATED CYTOGENETICS AND MOLECULAR ABNORMALITIES[1]

RISK STATUS	CYTOGENETICS	MOLECULAR ABNORMALITIES
Favorable-risk	Core binding factor: inv(16)[2,3] or t(16;16)[2] or t(8;21)[2] t(15;17)	Normal cytogenetics: NPM1 mutation in the absence of FLT3-ITD or isolated biallelic CEBPA mutation
Intermediate-risk	Normal cytogenetics +8 alone t(9;11) Other non-defined	t(8;21), inv(16), t(16;16): with c-KIT[5] mutation
Poor-risk	Complex (≥3 clonal chromosomal abnormalities) Monosomal karyotype -5, 5q-, -7, 7q- 11q23 - non t(9;11) inv(3), t(3;3) t(6;9) t(9;22)[4]	Normal cytogenetics: with FLT3-ITD mutation[6]

Appendix D: Contraceptive Guidelines and Pregnancy

Women Not of Childbearing Potential are Defined as Follows:

Women are considered post-menopausal and not of child bearing potential if they have had 12 months of natural (spontaneous) amenorrhea with an appropriate clinical profile (e.g. age appropriate, history of vasomotor symptoms) or six months of spontaneous amenorrhea with serum FSH levels > 40 mIU/mL [for US only: and estradiol < 20 pg/mL] or have had surgical bilateral oophorectomy (with or without hysterectomy) at least six weeks ago. In the case of oophorectomy alone, only when the reproductive status of the woman has been confirmed by follow up hormone level assessment is she considered not of child bearing potential.

Contraceptive Guidelines for Women of Child-Bearing Potential:

Women of child-bearing potential, defined as all women physiologically capable of becoming pregnant, must use highly effective contraception during the study and for 60 days after stopping treatment. The highly effective contraception is defined as either:

1. True abstinence: When this is in line with the preferred and usual lifestyle of the subject. Periodic abstinence (e.g., calendar, ovulation, symptothermal, post-ovulation methods) and withdrawal are not acceptable methods of contraception.

2. Sterilization: have had surgical bilateral oophorectomy (with or without hysterectomy) or tubal ligation at least six weeks ago. In case of oophorectomy alone, only when the reproductive status of the woman has been confirmed by follow up hormone level assessment.

3. Male partner sterilization (with the appropriate post-vasectomy documentation of the absence of sperm in the ejaculate). For female subjects on the study, the vasectomised male partner should be the sole partner for that patient.

4. Use of a combination of two of the following:

a) Barrier methods with spermicide:

- condom (male or female)
- occlusive cap (diaphragm or cervical/vault caps/shield)
- use of two barrier methods is acceptable (i.e. male condom + diaphragm or equivalent)

b) Placement of an intrauterine device (IUD) or intrauterine system (IUS).

c) Hormonal implants or combined oral contraceptives

- Please note it is unknown if vosaroxin may have the potential to decrease the effectiveness of hormonal contraceptives.

The following are unacceptable forms of contraception for women of childbearing potential:

- Natural family planning (rhythm method) or breastfeeding
- Fertility awareness
- Withdrawal

Appendix E. Prophylactic antimicrobials and mucositis care recommendations

Prophylactic antimicrobials

Patients should be on antimicrobial prophylaxis for duration of the immunocompromised periods during the trial. This connotes prophylaxis from diagnosis through count recovery (minimum ANC > 500 /µl or institutional practice; whichever is greater). There should be three lines of antimicrobial prophylaxis. These should include:

Antibacterial (e.g. Levafloxacin 500mg po daily)
Antifungal (e.g. Fluconazole 400mg po daily)
Antiviral (e.g. Valacyclovir 500mg po daily)

Empiric therapy for neutropenic fevers will be per institutional guidelines and will supercede needs for prophylactic antimicrobials.

Consistent with IDSA recommendations, patients with oral or gastrointestinal mucositis that interferes with swallowing or causes severe diarrhea as well as those with gastrointestinal symptoms, including abdominal pain, nausea and vomiting, or diarrhea should be considered at high-risk for serious complications in the setting of neutropenic fever and should receive empiric IV antibiotic therapy in the hospital setting.[38]

Additionally, severe mucositis in the setting of neutropenic fever should be considered an indication for the addition of antibiotics active against gram-positive organisms, if fluoroquinolone prophylaxis and empiric ceftazidime (or cefepime) have been employed.[38]

Mucositis care recommendations (extrapolated from MAASC Practice Guidelines)

Oral Mucositis

1. We recommend the use of oral cryotherapy on days 1 and 4 of vosaroxin infusions for mucositis prevention. Ice cubes / chips should be placed in patient's mouth starting 5 minutes prior to vosaroxin infusion and continuing for a total of 30 minutes duration.
2. We recommend patient controlled analgesia with morphine (or alternative narcotic pain medication as deemed appropriate per treating physician) be used to treat pain due to oral mucositis in patients receiving protocol defined chemotherapy.

   a. Alternative options
      i. Transdermal fentanyl, morphine mouthwash (0.2%), and doxepin mouthwash (0.5%) may also be effective to treat pain due to oral mucositis.
3. Zinc supplements administered orally may also be considered to prevent oral mucositis.
4. Oral care with salt and soda rinses or alternatives as per institutional practice are also allowed.
5. We recommend avoidance of prophylactic use of antimicrobial lozenges, antimicrobial mouthwash, sucralfate mouthwash, chlorhexidine mouthwash, GM-CSF mouthwash, misoprostol mouthwash, intravenous glutamine, systemic pentoxifylline, or systemic pilocarpine.

Gastrointestinal Mucositis

1. We recommend octreotide, at a dose of ≥100 µg subcutaneously twice daily, be used to treat diarrhea induced by protocol-defined chemotherapy if loperamide and/or lomotil are ineffective.
2. We recommend avoidance of systemic sucralfate, 5-acetyl salicylic acid (or related compounds), or misoprostol suppositories, to prevent or treat gastrointestinal mucositis.

Appendix F. Comorbidity Index and Risk Stratification Scoring Systems

Wheatley Score
Cytogenetic Group
WBC Count
ECOG Performance Status
Age
AML Subtype (De novo vs Secondary vs Unknown)

HCT-CI (www.hctci.org/Home/Calculator)
Arrythmia
Cardiovascular Comorbidity
Inflammatory Bowel Disease
Diabetes
Cerebrovascular Disease
Psychiatric Disturbance
Hepatic Comorbidity
Obestity Score
Infection
Rheumatologic Comorbidity
Peptic Ulcer
Renal Comorbidity
Pulmonary Score
Prior Solid Tumor
Heart Valve Disease
Age

AML-SCORE (www.aml-score.org)
Temperature
Hemoglobin
Platelets
Fibrinogen
LDH
Age at diagnosis
Type of Leukemia (de novo, secondary)
Cytogenetics