TITLE: Phase 1/2 Study of Liposomal Annamycin for the Treatment of Subjects with Acute Myeloid Leukemia (AML) that is Refractory to or Relapsed after Standard Induction Therapy

PROTOCOL NO. MB-104

IND Number 134 860

EudraCT number: 2017-002337-27

INVESTIGATIONAL DRUG: Liposomal Annamycin

SPONSOR:
Moleculin Biotech, Inc.
5300 Memorial Drive
Suite 950
Houston, TX, 77007
Telephone: 862-273-1432
Email: dpicker@moleculin.com

MONITOR:
Richard S. Ungerleider, MD
Senior Vice President, Clinical Research Affairs
Theradex
4365 Route 1, South Suite 101
Princeton, New Jersey 08540
Telephone: 609-799-7580 extension 3067
Mobile: 609-356-4072
Facsimile: 609-799-4148
Email: rungerleider@theradex.com

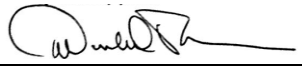
DATE OF VERSION: March 13, 2019

VERSION: 5.0

TABLE OF CONTENTS

PROTOCOL SIGNATURE SHEET

The undersigned have reviewed the format and content of this protocol and have approved Protocol No. MB-104 for issuance.



Sponsor Signature

March 13, 2019

Date

INVESTIGATOR SIGNATURE SHEET

I have read the attached protocol and agree that it contains all the necessary details for performing the study.

I will provide copies of the protocol and of the preclinical and clinical information on the test article, which was furnished to me by the Sponsor, to all members of the study team responsible to me who participate in the study. I will discuss this material with them to assure that they are fully informed regarding the test article and the conduct of the study.

Once the protocol has been approved by the Institutional Review Board (IRB)/Independent Ethics Committee (IEC), I will not modify this protocol without obtaining the prior approval of the Sponsor and of the IRB/IEC. I will submit the protocol modifications and/or any informed consent modifications to the Sponsor and the IRB/IEC, and approval will be obtained before any modifications are implemented.

I understand the protocol and will work according to the US FDA Code of Federal Regulations, the principles of Good Clinical Practice (current International Conference of Harmonisation [ICH] guidelines), European Clinical Trial Directive 2001/20/EC, European Union GCP Directive 2005/28/EC and the Declaration of Helsinki (1964) including all amendments up to and including the Fortaleza, Brazil, revision (2013).

_____ _____

Investigator's Signature Date

LIST OF ABBREVIATIONS

AE	adverse event
ALL	acute lymphocytic leukemia
AML	acute myeloid leukemia
BM	bone marrow
BUN	blood urea nitrogen
CABG	coronary artery bypass graft
CAD	coronary artery disease,
CBC	complete blood count
CNS	central nervous system
CR	complete response
CRi	CR with incomplete blood count recovery meets all criteria for CR except for neutropenia (<1,000; CRi) but must include transfusion independence
CRp	CR with incomplete blood count recovery meets all criteria for CR except for thrombocytopenia (<100,000; CRp) but must include transfusion independence
eCRF/EDC	Electronic Case Report Form/Electronic Data Capture
CTCAE	Common Terminology Criteria for Adverse Events
CV	cardiovascular
CYP	Cytochrome P450 family of enzymes
DLT	dose-limiting toxicity
DMSO	dimethyl sulfoxide
ECG	electrocardiogram
ECHO	echocardiogram
ECOG	Eastern Cooperative Oncology Group
EFS	event-free survival
EOS	end of study
EudraCT	European Union Drug Regulating Authorities Clinical Trials
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HSCT	hematopoietic stem cell transplantation
IEC	Independent Ethics Committee
ICH	International Conference of Harmonisation
IRB	Institutional Review Board
IV	intravenous(ly)
LDH	lactate dehydrogenase
LV	left ventricular
LVEF	left ventricular ejection fraction
LVH	left ventricular hyperthropy
MDR	multidrug-resistant
MI	myocardial infarction
MTD	maximum-tolerated dose
MUGA	multiple-gated acquisition
NCI	National Cancer Institute
OS	overall survival
PCI	percutaneous coronary intervention

PK	pharmacokinetic
PR	partial response
RP2D	recommended Phase 2 dose
SAE	serious adverse event
SGOT	serum glutamic-oxaloacetic transaminase
SGPT	serum glutamic-pyruvic transaminase
SRC	Safety Review Committee/Consultant
ULN	upper limit of normal
VHD	valvular heart disease

Summary of Changes

Page	Section	Description of Text
All	Headers	Versioning updated
Global	Global	Minor editorial changes were made throughout the document, including correction of grammatical and typographical errors, use of numeric forms, and addition of abbreviations.
Global	Global	NCI CTCAE v~~4.03~~ **5**
14, 16, 17, and 19	Protocol Synopsis Methodology	For each dose level, groups of 3 subjects will receive a treatment cycle of liposomal annamycin daily as a 2-hour intravenous (IV) infusion for 3 consecutive days **followed by 18 days off study drug (i.e., the induction treatment cycle = 21 days)**. If no subject experiences a dose-limiting toxicity (DLT), based on National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) v~~4.03~~ **5**, which constitutes a study drug-related irreversible or not medically manageable Grade 3 or higher toxicity~~, further defined in Section 3.4~~, during the induction treatment through ~~Day 42~~ **Day 28**, the subsequent group of 3 subjects will receive the next higher liposomal annamycin dose.
	Inclusion Criteria	8 Prior anthracycline cumulative dose ~~(see Table 2 and Appendix E) below 550~~ **<551** mg/m^2 or the daunorubicin equivalent, which is the recommended noncardiotoxic level. **b.** ~~Table 2 shows the maximal doses for other anthracyclines and the conversion factor for a daunorubicin equivalent is provided in Appendix E~~ **Potential cardioprotective measures for anthracycline drugs and analogues by limitation of cumulative doses are demonstrated by the following conversion factors:** **Daunorubicin = 1** **Doxorubicin = 2.2** **Epirubicin = 1.1** **Mitoxantrone = 5** **Idarubicin = 5.3** c. ~~Appendix E shows examples of how the maximal allowance based on daunorubicin equivalence is calculated~~ **The maximal allowance will be calculated as a daunorubicin equivalent added to the anticipated exposure to liposomal annamycin with the maximal exposure of anthracyclines capped at 550.9 mg/m^2 (~~Appendix E~~)** d. Exposure to annamycin projected for subjects enrolled in a given cohort in this study will be assessed on the basis of prior total exposure added to the anticipated designated cohort dose level (up to 120 mg/m^2, which, after 3 doses, representing a cumulative exposure of <~~550~~ **551-**mg/m^2 daunorubicin equivalents).
	Exclusion Criteria	5 ~~Prior anthracycline cumulative dose more than 50% above recommended noncardiotoxic levels,~~ **L**eft ventricular ejection fraction (LVEF) <50%, valvular heart disease, or severe hypertension ~~(see Table 1)~~
	Statistical Analyses	~~Study enrollment in the expansion phase portion of the study willbe halted if >2 of 6 or >3 of 10 subjects experience a DLT at the recommended Phase 2 dose~~. **The Medical Monitor and** the SRC will continually review all data and decide if the study should continue and if any changes to the protocol before restarting enrollment must be made.
24	1.6 Consideration	A value of ~~550~~ **<551** mg/m^2 will be used as the correlative value for

	of Cardiotoxicity in the Current Study	liposome-encapsulated annamycin for purposes of assessment of maximal exposure, with a conversion factor of 1.
25-29	3.1 Design Summary	For each dose level, groups of 3 subjects will receive a treatment cycle of liposomal annamycin daily as a 2-hour intravenous (IV) infusion for 3 consecutive days **followed by 18 days off study drug (i.e., the induction treatment cycle = 21 days).** If no subject experiences a dose-limiting toxicity (DLT), based on National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) v~~4.03~~ **5**, which constitutes a study drug-related irreversible or not medically manageable Grade 3 or higher toxicity, further defined in Section 3.4, during the induction treatment through ~~Day 42~~ **Day 28**, the subsequent group of 3 subjects will receive the next higher liposomal annamycin dose. ~~Study enrollment in the expansion phase portion of the study willbe halted if >2 of 6 or >3 of 10 subjects experience a DLT at the recommended Phase 2 dose~~. **The Medical Monitor and** the SRC will **continually** review all data and decide if the study should continue and if any changes to the protocol before restarting enrollment must be made.
	3.2 Dosing	The initial group of 3 subjects will be treated with a 2-hour intravenous infusion of 100 mg/m^2/day (Dose Level 1) liposomal annamycin daily for 3 consecutive days followed by 18 days off study drug (i.e., **1** treatment cycle = 21 days). Provided that no subject experiences a DLT (defined in Section 3.4) during the induction treatment cycle through ~~Day 42~~ **Day 28**, the ensuing group of 3 subjects will receive 120 mg/m^2/day (Dose Level 2) liposomal annamycin-
	3.3.1 Cardiotoxicity	Subjects will also have clinical and electrocardiographic examinations evaluated at the anticipated maximal plasma concentrations, and again weekly during the induction cycle of treatment with multiple-gated acquisition (MUGA) or echocardiogram (ECHO **[2-dimensional accepted, 3-dimensional preferred]**) scans, or ECHO strain evaluations performed every 3 weeks by using the same modality and instrument. Any subject demonstrating any of the above manifestations of cardiotoxicity, or >10% decrease in ejection fraction based on scans, will be discontinued from the study. **Between the Day 18 and Day 28 visits or at the End of Treatment visit (if the subject is prematurely terminated)**, a final MUGA or ECHO scan~~,~~ or **an** ECHO strain evaluation ~~,~~ ~~using the same modality and instrument,~~ will be conducted **by using the same modality and instrument**~~one week after the treatment cycle or after the last study drug administration, if the treatment period is prematurely terminated~~
	3.4 Criteria for Dose-Limiting Toxicity	The DLT evaluation period is defined as the first day of treatment (Day 1) until ~~Day 42~~ **Day 28.** ~~The following hematologic toxicities that occur after the cycle of treatment that do not resolve by Day 42 will be considered a DLT:~~ • ~~•Peripheral absolute neutrophil count (ANC) <500/mm^3 (Grade ≥4);~~ • ~~•Non-transfusion-dependent platelet count <20,000/mm^3 due to documented bone marrow aplasia/hypoplasia (vs. malignant infiltration or other cause). Bone marrow aplasia/hypoplasia is defined as overall marrow cellularity <20%;~~ • ~~Platelet count <50,000/mm^3 (Grade ≥3) that is associated with~~

		~~bleeding;~~ ~~Platelet count <25,000/mm^3 (Grade 4) that requires platelet transfusion.~~
	3.5.1 Bone Marrow Evaluation	Bone marrow aspirate (a biopsy if there are no spicules present) ~~14-21~~ **15 to 35** days after the start of therapy will be performed**.** ~~to document hypoplasia. If hypoplasia is not documented or indeterminate, a repeat biopsy should be obtained in another 7-14 days to clarify the persistence of leukemia. If hypoplasia is present, then a repeat biopsy at the time of hematologic recovery based on peripheral blood counts should be obtained to document remission.~~
31, 32, and 33	4.1 Inclusion Ctiteria	8 Prior anthracycline cumulative dose (see Table 2 and Appendix E) ~~below 550~~ <**551** mg/m^2 or the daunorubicin equivalent, which is the recommended noncardiotoxic level. **c. Potential cardioprotective measures for anthracycline drugs and analogues by limitation of cumulative doses are demonstrated by the conversion factors in** Table 2 ~~shows the maximal doses for other anthracyclines and the conversion factor for a daunorubicin equivalent is provided in Appendix E~~ d. ~~Appendix E shows examples of how the maximal allowance based on daunorubicin equivalence is calculated~~ **The maximal allowance will be calculated as a daunorubicin equivalent added to the anticipated exposure to liposomal annamycin with the maximal exposure of anthracyclines capped at 550.9 mg/m^2 (Appendix E)** e. Exposure to annamycin projected for subjects enrolled in a given cohort in this study will be assessed on the basis of prior total exposure added to the anticipated designated cohort dose level (up to 120 mg/m^2, which, after 3 doses, representing a cumulative exposure of <~~550~~ **551-**mg/m^2 daunorubicin equivalents).
	4.2 Exclusion Criteria	5 ~~Prior anthracycline cumulative dose more than 50% above recommended noncardiotoxic levels,~~ LVEF <50%, valvular heart disease, or severe hypertension (see Table 1)
	4.3.2 Subject Replacement	Subjects withdrawing in the cohort dose-escalation period for reasons other than toxicity who have not taken all 3 doses during the induction cycle and/or fail to complete restaging after completion of the treatment cycle ~~(~21 days)~~ such that safety ~~or efficacy~~ is not evaluable will be replaced. However, subjects experiencing a DLT in the dose-finding cycle will not be replaced.
34	5.1.2 Preparation of Liposomal Annamycin	The study drug must be reconstituted prior to use. The diluted product can be held for up to ~~4 hours~~ **24 hours** when stored between ~~25 to 37ºC~~ **34 to 42ºC**, after which time unused drug should not be administered to subjects.
39-40	6.3.2 Induction Cycle	In addition to the weekly evaluations during the induction cycle, the following evaluations will be conducted at the end of the 3-week treatment cycle ~~(between Days 14-21)~~: ~~1. Physical examination including weight, vital signs, and oral exam~~ 1. MUGA or ECHO scan or ECHO strain evaluation **between Days 18 and 28**. Cardiology consultation should be requested if any question arises about cardiac function. **The MUGA scan should also be conducted at the Early Termination visit for subjects who are withdrawn prematurely.** 2. Bone marrow aspirate and peripheral blood specimens (complete blood count, differential, and platelet count; flow cytometry is optional) between **Days 15 and 35** to document response
	6.4 End of Study	

	Evaluations 6.5 Follow-up Evaluations 6.6. Survival Follow-up	~~1. Physical examination including weight, vital signs, and oral exam~~ 2. ~~CBC with differential and platelet count, if subject had a hematological toxicity which could be classified as a DLT (as defined in Section 3.4)~~ Subjects will be followed every 3 months from study drug discontinuation to monitor resolution or stabilization of any treatment-related medical events and obtain survival data. **If possible, a MUGA scan, ECHO scan, or ECHO strain evaluation will be collected.**
42	7.2 Methods for Measuring Efficacy	Bone marrow aspirate/biopsy and peripheral blood specimens (complete blood count, differential, and platelet count) to document response will be collected at ~~14-21 days~~ **15 to 35 days** after the start of therapy~~ to document hypoplasia. If hypoplasia is not documented or indeterminate, a repeat biopsy should be obtained in 7-14 days to clarify the persistence of leukemia. If hypoplasia is present, then a repeat biopsy at time of hematologic recovery should be obtained to document remission~~.
47	10. Statistics	~~Study enrollment in the expansion phase portion of the study will be halted if >2 of 6 or >3 of 10 subjects experience a DLT at the recommended Phase 2 dose.~~ **The Medical Monitor and** the SRC will **continually** review all data and decide if the study should continue and if any changes to the protocol before restarting enrollment must be made.
54-56	Appendix A: Schedule of Events	Day 21 ~~±3~~ (At the End of the Induction Cycle) CBC, differential, platelet count[c,d] Insertion of columns for Day 15, Day 18, and Day 35 Day 42 CBC, differential, platelet count: ~~X~~[e] Day 18 to 28 MUGA, ECHO, or ECHO strain: **X** Survival Follow-up: MUGA, ECHO, or ECHO strain: **X[q]** Day 15 to 35 Bone marrow aspirate and peripheral blood[h] for measuring disease: **X** **[g] A final MUGA or ECHO scan or an ECHO strain ealuation will be conducted between the Day 18 and Day 28 visits or at the the End of Treatment visit if the subject is prematurely terminated.** Footnotes d, e, f, g, h, i, j, k, l, m, n, and o were renumbered to e, f, h, i, j, k, l, m, n, o, p, and d, respectively. ~~f~~**[h]** Bone marrow aspirate and peripheral blood (complete blood count, differential, and platelet count; flow cytometry is optional) within 15 days prior to the first dose of liposomal annamycin to document disease status. Bone marrow aspirate/biopsy ~~14-21~~ **15 to 35** days after the start of therapy will be performed**.** ~~to document hypoplasia. If hypoplasia is not documented or indeterminate, a repeat biopsy should be obtained in another 7-14 days to clarify the persistence of leukemia. If hypoplasia is present, then a repeat biopsy at the time of hematologic recovery based on peripheral blood counts should be obtained to document remission.~~ If cytogenetics were initially abnormal, include cytogenetics as part of the remission documentation. **[q] A MUGA scan, ECHO scan, or ECHO strain will be collected at the Q3-**

		month survival timepoint if possible.
59	Appendix E Calculations of Anthracycline Exposure	All daunorubicin equivalents are based on ~~550~~ <**551** mg/m^2 as being the maximal allowable dose **with the maximal exposure of anthracyclines capped at 550.9 mg/m^2.** ~~Example calculations:~~ ~~**A subject with prior FLA(G) IDA Induction (Idarubicin 24 mg/m^2) for 1 course:**~~ ~~24 mg/m^2 per course~~ ~~24 × 5.3 (conversion factor of daunorubicin = 127. 2 mg/m^2: daunorubicin equivalents~~ ~~With maximal exposure of anthracyclines based on daunorubicin equivalent capped at 550 mg/m^2:~~ ~~550 mg/m^2 – 127.2 mg/m^2 = 422.8 mg/m^2:~~ ~~Therefore, additional liposomal annamycin of no more than 422.8 422.8 mg/m^2 exposure will be permitted (subject allowed to receive 3 doses at 120 mg/m^2)~~ ~~**A subject with prior D(60)A 3+7 Induction (Daunorubicin 180 mg/m^2)**~~ ~~With maximal exposure of anthracyclines based on daunorubicin equivalent capped at 550 mg/m^2:~~ ~~Daunorubicin 550 mg/m^2 – 180 mg/m^2 = 370 mg/m^2~~ ~~Therefore, additional liposomal anthramycin of no more than 370 mg/m^2 exposure is permitted (subject allowed to receive 3 doses at 120 mg/m^2)~~

PROTOCOL SYNOPSIS

Protocol No.:	MB-104
Protocol Title:	Phase 1/2 Study of Liposomal Annamycin for the Treatment of Subjects with Acute Myeloid Leukemia (AML) that is Refractory to or Relapsed after Standard Induction Therapy
Clinical Phase:	Phase 1/2
Investigator(s):	TBD
Study Center(s):	Approximately 7
Name of Sponsor/ Company:	Moleculin Biotech, Inc.
Objectives:	The primary objective of this study is to evaluate the safety and identify the recommended Phase 2 dose (RP2D) of liposomal annamycin for the treatment of subjects with AML that is refractory to or relapsed after standard induction therapy. Secondary objectives: 1. Pharmacokinetics of annamycin and its metabolite, annamycinol 2. Preliminary assessment of the antileukemic activity of liposomal annamycin as second line therapy for subjects with refractory or relapsed AML based on established response criteria, including complete response (CR), partial response (PR), event-free survival (EFS), overall survival (OS; Kaplan-Meier), and time to and duration of remission/response
Patient Population:	Subjects diagnosed with AML that is refractory to or relapsed after standard induction therapy.
Methodology:	This is a multicenter, open-label, dose-escalation study that will determine the maximum-tolerated dose (MTD) and RP2D of liposomal annamycin as a single agent for the treatment of subjects with AML that is refractory to or relapsed after standard induction therapy. During the dose-escalation phase of the study, eligible subjects could have received any number of previous therapies for their relapsed AML as long as the prior anthracycline cumulative dose is below the recommended noncardiotoxic level). During the expansion phase of the study, after the MTD or RP2D has been established, study treatment will be second-line therapy, thus subjects could not receive prior therapy for their relapsed AML. Enrollment will occur in cohorts of 3 subjects in a conventional 3+3 escalating dose design, starting at a dose level of 100 mg/m^2/day administered for 3 days. Dose escalation will take place on the basis of safety assessments in this first cohort with the next dose of liposomal annamycin escalated to 120 mg/m^2 in the absence of safety concerns. For each dose level, groups of 3 subjects will receive a treatment cycle of liposomal annamycin daily as a 2-hour intravenous infusion for 3 consecutive days followed by 18 days off study drug (i.e., the induction treatment cycle = 21 days).. If no subject experiences a dose-limiting toxicity (DLT), based on National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) v5, which constitutes a study drug-related irreversible or not medically manageable Grade 3 or higher toxicity during the induction treatment through Day 28, the subsequent group of 3 subjects will receive the next higher liposomal annamycin dose. However, if 1 of the 3 initial subjects experiences a DLT, the cohort of subjects at that dose level will be expanded to 6 subjects. If at least 2 of the 6 subjects experience a DLT, this will be considered a nontolerated dose and then 3 subjects will be treated at a lower dose.

	The MTD is defined as the highest dose of liposomal annamycin at which fewer than 2 (of a cohort of up to 6) subjects experience a DLT. The RP2D is defined as the optimal dose to be determined by the Sponsor in conjunction with an independent Safety Review Committee/Consultant (SRC), consisting of at least 1 independent consultant, on the basis of review of available clinical and laboratory safety and efficacy data. Up to 21 additional subjects will be enrolled at either the MTD or RP2D, to better define toxicity and evaluate efficacy at this dose. If a subject discontinues treatment for reasons other than study drug-related adverse events such that safety and efficacy of the drug cannot be fully evaluated during the induction cycle, a replacement subject may be enrolled; these circumstances will be reviewed on a case-by-case basis by the SRC in conjunction with the Sponsor.
Number of Subjects:	Three subjects minimum (6 maximum) for each dosing cohort. Evaluation of a RP2D based on the aggregate assessment of data will be determined by the SRC in conjunction with the Sponsor, with consideration of additional dose exploration based on safety assessments. Up to 21 additional subjects will be enrolled at the MTD or RP2D to better define toxicity and evaluate efficacy at this dose. Therefore up to 33 subjects will be enrolled in this study: up to 12 subjects in the dose-escalation phase and up to 21 additional subjects at the MTD or RP2D.
Study Drug:	Annamycin is a lipophilic anthracycline antibiotic that incorporates 4 structural modifications from doxorubicin: 2'-iodo, 3'-hydroxy, 4'-epi, and 4-demethoxy doxorubicin. Liposomal annamycin is supplied in 50-mL vials containing annamycin as a lyophilized powder. Subjects will be treated daily for 3 consecutive days with a 2-hour intravenous infusion of liposomal annamycin followed by 18 days off liposomal annamycin (i.e., 1 treatment cycle = 21 days). The starting dose of liposomal annamycin (dose for subjects in Cohort 1) is 100 mg/m^2/day. The liposomal annamycin doses will be escalated up to 120 mg/m^2/day in the next cohort in the absence of safety concerns.

Inclusion Criteria:	**Inclusion Criteria** 1. A pathologically confirmed diagnosis of AML by World Health Organization classification 2. AML that is refractory to or relapsed after standard induction therapy 3. Age ≥18 years at the time of signing informed consent 4. No chemotherapy, radiation, or major surgery within 2 weeks prior to first dose of study drug and/or recovered from the toxic side effects of that therapy, unless treatment is indicated due to progressive disease 5. No investigational therapy within 4 weeks of the first dose of study drug 6. Eastern Cooperative Oncology Group (ECOG) performance status 0 to 2 7. Adequate laboratory results including the following: a. Bilirubin ≤1.5 times the upper limit of normal unless due to Gilbert Syndrome b. Serum glutamic-oxaloacetic transaminase , serum glutamic-pyruvic transaminase, and alkaline phosphatase <3 times the upper limit of normal) unless due to organ involvement c. Adequate renal function (The Cockroft-Gault equation will be used to estimate creatinine clearance. This equation is as follows: Creatinine clearance in milliliters per minute = [140 – age] × body weight [kg]/72 × plasma creatinine [mg/dL], multiplied by 0.85 for women. By using this equation, adequate renal function will be deemed to be a creatinine clearance of greater than 60 mL/minute.) 8. Prior anthracycline cumulative dose <551 mg/m^2 or the daunorubicin equivalent, which is the recommended noncardiotoxic level. This will be calculated as follows: a. Determination of prior exposure will be based on total milligrams per meter squared of prior anthracycline therapy). b. Potential cardioprotective measures for anthracycline drugs and analogues by limitation of cumulative doses are demonstrated by the following conversion factors: Daunorubicin = 1 Doxorubicin = 2.2 Epirubicin = 1.1 Mitoxantrone = 5 Idarubicin = 5.3 c. The maximal allowance will be calculated as a daunorubicin equivalent added to the anticipated exposure to liposomal annamycin with the maximal exposure of anthracyclines capped at 550.9 mg/m^2. d. Exposure to annamycin projected for subjects enrolled in a given cohort in this study will be assessed on the basis of prior total exposure added to the anticipated designated cohort dose level (up to 120 mg/m^2, which, after 3 doses, representing a cumulative exposure of <551-mg/m^2 daunorubicin equivalents). 9. Subject can understand and sign the informed consent document, can communicate with the Investigator, and can understand and comply with the requirements of the protocol. 10. Women of childbearing potential must have a negative serum or urine pregnancy test. 11. All men and women must agree to practice effective contraception during the entire study period and after discontinuing study drug, unless documentation of infertility exists. a. Sexually active, fertile women must use 2 effective forms of contraception (abstinence, intrauterine device, oral contraceptive, or double barrier device) from the time of informed consent and until at least 6 months after discontinuing study drug

	b. Sexually active men and their sexual partners must use effective contraceptive methods from the time of subject informed consent and until at least 3 months after discontinuing study drug
Exclusion Criteria:	**Exclusion Criteria** 1. Subjects diagnosed with acute promyelocytic leukemia 2. Concomitant therapy that includes other chemotherapy that is or may be active against AML except for prophylaxis and/or treatment of opportunistic or other infection with antibiotics, antifungals, and/or antiviral agents 3. Prior mediastinal radiotherapy 4. Any condition that, in the opinion of the Investigator, places the subject at unacceptable risk if he/she were to participate in the study. 5. Left ventricular ejection fraction (LVEF) <50%, valvular heart disease, or severe hypertension. Cardiac subjects with a New York Heart Association classification of 3 or 4 will be excluded. (Cardiology consultation should be requested if any question arises about cardiac function.) This also includes subjects with baseline QT/QTc interval >480 msec, a history of additional risk factors for torsade des pointes (e.g., heart failure, hypokalemia, family history of Long QT Syndrome), and use of concomitant medications that significantly prolong the QT/QTc interval. 6. Clinically relevant serious comorbid medical conditions including, but not limited to, active infection, recent (less than or equal to 6 months) myocardial infarction, unstable angina, symptomatic congestive heart failure, uncontrolled hypertension, uncontrolled cardiac arrhythmias, chronic obstructive or chronic restrictive pulmonary disease, active central nervous system disease uncontrolled by standard of care, known positive status for human immunodeficiency virus and/or active hepatitis B or C, cirrhosis, or psychiatric illness/social situations that would limit compliance with study requirements 7. Pregnant, lactating, or not using adequate contraception 8. Known allergy to anthracyclines 9. Any evidence of mucositis/stomatitis or previous history of severe (≥Grade 3) mucositis from prior therapy 10. Required use of strong inhibitors and inducers of Cytochrome P450 family of enzymes (CYP) and transporters that cannot be held during treatment days
Criteria for Evaluation: (Safety):	1. Clinical adverse events (AEs), routine hematological and biochemical parameters, physical examination and vital signs, 12-lead electrocardiograms (ECGs), ECOG performance status, pregnancy testing, and monitoring of concomitant medications will be assessed according to the Schedule of Events. 2. All AEs and laboratory abnormalities will be graded for severity by using the NCI CTCAE v5. The relatedness of all AEs and laboratory abnormalities and their seriousness will be determined by the Investigator and adjudicated by the SRC in conjunction with the Sponsor. The SRC will consist of an external, independent (not participating in the clinical trial) committee/consultant providing guidance with regard to safety issues, including approval of dose escalation and designation of DLTs. 3. AEs will be documented from the time of first dose of study drug until 39 days after the last dose administered on study. 4. Strategies for detection of cardiotoxicity that include cardiac imaging (echocardiography, nuclear imaging, cardiac magnetic resonance) and biomarkers (troponin, natriuretic peptides) will be based on clinical assessments at screening, as well as current relevant guidelines

Criteria for Evaluation (Efficacy):	All subjects evaluable for efficacy will be assessed for response to treatment by using the recommendations of the International Working Group for standardization of response criteria, treatment outcomes, and reporting for therapeutic trials. The primary efficacy variable is leukemia response rate, evaluated by the Investigator at the end of the induction cycle and at the end of study, based on bone marrow aspirate and peripheral blood collected at the end of the induction cycle. A bone marrow (BM) aspirate/biopsy should be repeated in 1 week if there is a question of residual leukemia in assessing efficacy based on an initial BM specimen. Primary Efficacy Endpoints: 1. Morphologic leukemia-free state a. BM <5% blasts in an aspirate with spicules (a BM biopsy should be performed if spicules are absent) b. No blasts with Auer rods or persistence of extramedullary disease 2. Complete remission: a. Morphologic leukemia-free state in which i. Subject is independent of transfusions ii. Absolute neutrophil count of >1,000 iii. Platelets of ≥100,000 3. Cytogenetic complete response – cytogenetics normal in those with previously abnormal cytogenetics 4. CRi and CRp: CR with incomplete blood count recovery meets all criteria for CR except for either neutropenia (<1,000; CRi) or thrombocytopenia (<100,000; CRp) but must include transfusion independence Secondary Endpoints: 1. EFS: Time from enrollment until disease progression or death from any cause 2. OS: Time from enrollment until death from any cause 3. PR: All of the hematologic values for a CR, with normalization of blood counts, but with a decrease of at least 50% in the percentage of blasts to 5% to 25% in the bone marrow aspirate 4. Time to and duration of remission/response
Pharmacokinetic Analyses:	Pharmacokinetics of annamycin and its metabolite, annamycinol, will be measured after the first dose of liposomal annamycin on Day 1 and Day 3 of the induction cycle. Blood samples for pharmacokinetic analysis will be collected on these days at predose and at 0.25, 0.5, 1, 2, 4, 8, and 24 hours after the start of liposomal annamycin infusion for 3 subjects at each dose and 6 subjects at the RP2D. ECGs and documentation of concomitant medications potentially affecting CYP enzymes will be obtained to ensure that these are a consideration in the evaluation of the safety and pharmacokinetics of this drug.
Statistical Analyses	This is a multicenter, open-label, dose-escalation, MTD/RP2D safety study of liposomal annamycin given to patients with refractory or relapsed AML. Therefore, only descriptive statistics will be utilized. A maximum of 33 patients will be recruited for the study depending on the level at which toxicity is observed. Data from all subjects who receive 1 or more doses of liposomal annamycin will be incorporated into the final safety analysis. Safety and tolerability will be assessed by adverse events, vital signs, physical examination, laboratory parameters, and an ECHO or multiple-gated acquisition (MUGA) scan or an ECHO strain evaluation, as well as cardiotoxicity biomarkers. Adverse events will be listed by subject and

	will be tabulated and summarized as the number and percentage of subjects who reported each adverse event. Descriptive statistics will be generated as appropriate; otherwise, no formal statistical analyses are planned. The Medical Monitor and the SRC will continually review all data and decide if the study should continue and if any changes to the protocol before restarting enrollment must be made.

1. BACKGROUND

1.1 Acute Myeloid Leukemia

Adult acute myeloid leukemia (AML) is the most common type of leukemia in adults, accounting for approximately 25% of leukemia diagnoses, with an estimated 19,950 new cases and an estimated 10,430 patients will die from AML this year in the US (NCI 2016; Deschler et al. 2006). The 5-year survival rate in patients with AML is approximately 25%. AML evolves rapidly and develops resistance to therapy because of its polyclonal and heterogeneous features (Ferrara et al. 2013). Cytogenetic and molecular mutation testing may be important for risk assessment and prognosis, but they are not predictive. So far, ascertaining a specific mutation does not predict what therapy is likely to work (Patel et al. 2012).

AML patients initially respond well to the decades' old and still standard 3 + 7 induction regimen if they are candidates for this intensive therapy. Response and survival are associated with multiple factors, including patient age, performance status, antecedent hematologic conditions (de novo vs. secondary AML), and cytogenetic and molecular features. Responses in the second line of therapy rely on these prognostic factors but also include the achievement of minimal residual disease status and the duration and type of response, e.g., complete response (CR) or CRi (complete response with incomplete bone marrow recovery) in the first line (Chen et al. 2015). Patients in first relapse with a first CR duration of 12 months or longer are offered high-dose cytarabine-based regimens. These include fludarabine with cytarabine and idarubicin, clofarabine with idarubicin and cytarabine, cladribine with idarubicin and cytarabine, twice daily fludarabine, cytarabine, or high-dose cytarabine-based investigational approaches. For patients in first salvage with a first CR duration <12 months and all patients in second salvage or beyond, Phase 1 to 2 investigational approaches are reasonable (Kantarjian 2016).

1.2 Liposomal Annamycin

Doxorubicin is one of the most effective antitumor agents against hematological malignancies, including AML, and certain human solid tumors such as breast carcinoma and osteosarcoma. Its use is limited by myelosuppression, chronic cardiotoxicity, and natural or acquired drug resistance. Important advances in understanding the mechanisms of acquired resistance to doxorubicin and other structurally unrelated antitumor agents have occurred recently. Overexpression of a membrane glycoprotein, P-glycoprotein, which acts as a drug efflux pump, mediates acquired resistance to doxorubicin in some *in vitro* systems and *in vivo* animal tumor models (DeVita et al. 1993). Overexpression of P-glycoprotein occurs in a significant number of patients when their tumors progress or relapse after treatment with doxorubicin. No standard regimen exists for the treatment of patients with relapsed AML, particularly in patients with first remission duration of less than 1 year.

Extensive efforts have been devoted to the synthesis of anthracycline analogs with improved properties. Initial efforts were directed towards analogs with reduced cardiotoxicity (Dalton et al. 1993; Moscow et al. 1988; Consoli et al. 1996). More recently, triggered by the discovery of multidrug resistance and identification of P-glycoprotein, the efforts have focused on analogs with non-cross-resistant properties (Acton et al. 1984; Ganapathi et al. 1989; Barbieri et al. 1987; Priebe et al. 1993). Several non-cross-resistant analogs have been identified. They all have in common a markedly increased lipophilicity. Some of them have a similar mechanism of cytotoxicity to that of doxorubicin, i.e., topoisomerase II inhibition. Others have a different mechanism of action, i.e., DNA alkylation (Acton et al. 1984).

Liposomes have been used extensively as carriers of doxorubicin and daunorubicin (Gabizon et al. 1982; Rahman et al. 1986). Liposomal doxorubicin was found to be less cardiotoxic and more active than doxorubicin in models of liver metastases in mice (Mayhew et al. 1983; Herman et al. 1983). Clinical studies have been conducted with different liposomal formulations of doxorubicin. These trials have shown a maximum-tolerated dose (MTD) similar to that of free doxorubicin, a significant reduction of certain toxicities, such as gastrointestinal and vesicant effects, and an unchanged dose-limiting toxicity, i.e., myelosuppression. These studies have also suggested a reduced cardiotoxic potential.

Annamycin is an anthracycline antibiotic with a mechanism of action that appears to be inhibition of topoisomerase-II. This drug was selected for its lack of cross-resistance and a high affinity for lipid membranes (Perez-Soler et al. 1990). Because of this latter property, annamycin is an ideal compound for liposome entrapment, thus as this drug is completely insoluble in water solutions, liposomes can be used as a carrier for its intravenous administration. Liposomal annamycin was developed to combine the intrinsic favorable properties of the compound (lack of cross-resistance) with the potential advantages associated with liposome delivery (reduced cardiotoxicity and preferential distribution to certain organs), as well as the potential for resisting multidrug-resistant (MDR) activity (Perez-Soler et al. 1997; Kolonias et al. 1999). A liposomal formulation of annamycin was developed at MD Anderson Cancer Center that has high entrapment efficiency, physical stability, and chemical stability.

1.3 Nonclinical Studies

The *in vitro* cytotoxicity of liposomal annamycin was tested on a panel of 4 different parental cell lines and their respective MDR-1-expressing cell lines (Zou et al. 1994). In all MDR cell lines tested (KB-V1, P388/Dox, CEM/Vbl, and 8226/R), there was minimal resistance to annamycin; however, as expected, resistance to doxorubicin was high. Studies with MDR modulators and metabolic inhibitors indicated significant differences in the cellular transmembrane transport systems between doxorubicin and annamycin and suggest that annamycin efflux is not mediated by P-glycoprotein MDR (Perez-Soler et al. 1994).

In vivo, liposomal annamycin has shown lack of cross-resistance in KB-VI human xenografts (an MDR-resistant cell line) and enhanced antitumor activity compared with doxorubicin in several mouse tumor models such as leukemia (L-1210), melanoma (B16), reticulosarcoma (M5076), and Lewis lung carcinoma cells (Zou et al. 1994; Perez-Soler et al. 1990). Results in KB and KB-V1 human xenografts demonstrate that liposomal annamycin was at least as effective as doxorubicin (Zou et al. 1994). Liposomal annamycin was more active than doxorubicin in a leukemia L--1210 mouse tumor model (Perez-Soler et al. 1990).

In mice, at a single intravenous dose of 15.7-mg/kg liposomal annamycin, myelosuppression was noted. With weekly intravenous doses of 5.2-mg/kg liposomal annamycin for 6 weeks or 3.1- and 4.2-mg/kg liposomal annamycin for 10 weeks, the cardiotoxicity of liposomal annamycin was less than equitoxic doses of doxorubicin (Zou et al. 1995). In dogs, a single 15-minute intravenous infusion of up to 1.42-mg/kg liposomal annamycin was well tolerated, with no clinically significant adverse effects, hematological and chemical changes, and pathological changes (Zou et al. 1995).

Additional information about the nonclinical data with liposomal annamycin can be found in the Investigator's Brochure.

1.4 Clinical Studies

A Phase 1, open-label, dose-escalating clinical study was conducted in subjects with refractory or relapsed AML, myelodysplastic syndrome, or acute lymphocytic leukemia (ALL) (Study No. AR522-21) (Andreeff et al. 2001). The study was initiated in March 1999 and completed in November 2000. Cohorts of 3 subjects were to be treated, with a starting dose for the first cohort of 190 mg/m^2/day intravenously (IV) for 3 consecutive days. Dosing for additional cohorts was on an escalating basis (230-, 280-, and 350-mg/m^2/day IV liposomal annamycin for 3 consecutive days). Therapy was to be repeated every 4 to 6 weeks.

Twenty subjects were treated: 3 at 190 mg/m^2/day, 2 at 230 mg/m^2/day, 2 at 280 mg/m^2/day, and 13 at 350 mg/m^2/day. Subjects received up to 3 cycles of therapy. An encouraging response was obtained in a subject with refractory ALL. Specifically, 1 out of 3 ALL subjects treated had a complete remission. This subject had 3 relapses prior to treatment with liposomal annamycin, and was treated with 350-mg/m^2/day liposomal annamycin for 1 cycle and 280 mg/m^2/day liposomal annamycin for 1 cycle. The duration of response was 5 weeks. An encouraging response was also obtained in a subject with refractory AML. Specifically, 1 out of the 16 AML subjects treated had a complete remission. This subject had failed induction therapy with CAT, and was treated with 280-mg/m^2/day liposomal annamycin for 1 cycle and 210 mg/m^2/day for 2 cycles. The duration of response was 13 weeks.

A Phase 1/2, open-label, dose-escalating clinical study was conducted in subjects with refractory or relapsed ALL (Protocol No. CP-103; NCT00271063) (Wetzler et al. 2013). The study was initiated in December 2005 and was completed in November 2008. The starting annamycin dose was 190 mg/m^2/day as a 2-hour intravenous infusion for 3 consecutive days followed by 18 days without study drug (treatment cycle equaled 21 days).

A total of 31 subjects were treated. The MTD was determined to be 150 mg/m^2/day for 3 days. Other than tumor lysis syndrome, which developed in 2 subjects, 3 severe adverse events (AEs) considered definitely related to the study drug consisted of Grade 3 mucositis, which defined the MTD. There was also 1 case each of Grade 3 diarrhea, typhlitis, and nausea. Left ventricular ejection fractions (LVEFs) were unchanged after 1 course of treatment. Four subjects received more than 1 course of treatment; LVEF decreased in 2 of them (from 50%-55% to 38% in 1 and from 50%-55% to 35% in the other) after the second course of treatment and stayed the same in the other 2 subjects. Two additional subjects who received only 1 course of annamycin had late LVEF determinations that were unchanged.

After the MTD was determined, 10 subjects were enrolled in the Phase 2 section of the trial. Eight of the subjects completed 1 cycle of the 3 days of treatment at the MTD. Of these, 5 (62%) had an efficacy signal with complete clearing of circulating peripheral blasts. Three of these subjects also cleared bone marrow blasts with 1 subsequently proceeding on to successful stem cell transplantation. The other 2 developed tumor lysis syndrome and died before response assessment.

In addition to the 2 clinical trials in leukemia subjects, liposomal annamycin has been studied in 2 clinical trials to determine the safety and efficacy in subjects with relapsed solid tumors, doxorubicin-resistant breast cancer, and metastatic breast cancer (NCT00012129) (Booser et al. 2002; Booser et al. 2000). Doses in these 2 studies ranged from 3- to 240-mg/m^2 IV liposomal annamycin given every 21 days.

Additional information about the previous human experience with liposomal annamycin can be found in the Investigator's Brochure.

1.5 Rationale for the Current Study

Based on the positive clinical results in subjects with acute leukemia in 2 previous clinical studies of single-agent liposomal annamycin (Study No. AR522-21 and Study No. CP-103; see Section 1.4), the nonclinical data showing both lack of cross-resistance with anthracyclines (which is the standard of care for first-line treatment of AML), the increased activity in certain leukemia cells lines compared with doxorubicin, and the potential for decreased cardiotoxicity, the current study (Study No. MB-104) was designed to explore liposomal annamycin in a Phase 1/2 investigation for the treatment of relapsed/refractory AML. Safety, pharmacokinetics, and clinical response will be evaluated. The objective of the study is to determine the safety and tolerability of annamycin, identify dose-limiting toxicities, and define the MTD, as well as the recommended Phase 2 dose (RP2D). The design of this study incorporates a 3+3 dose-escalation design and expansion at the RP2D for evaluation of clinical efficacy in up to 21 additional subjects.

1.6 Consideration of Cardiotoxicity in the Current Study

On the basis of concerns about the possible risk of cardiotoxicity in subjects who have likely received prior anthracycline therapy (Zamorano et al. 2016; Gianni et al. 2008), Table 1 and Table 2 establish some important considerations, including baseline risk factors and maximal

prior anthracycline doses, to be calculated on the basis of equivalent anthracycline exposure anticipated. This protocol incorporates clear criteria for risk factors, as well as prior anthracycline exposure, to exclude subjects at cardiac risk from this study.

Table 1: Baseline Risk Factors for Cardiac Disease (Zamorano et al. 2016)

Current Myocardial Disease:
Heart failure (with either preserved or reduced ejection fraction)
Asymptomatic LV dysfunction (LVEF <50% or high natriuretic peptide)
Evidence of CAD (previous MI, angina, PCI or CABG, myocardial ischemia)
Moderate and severe VHD with LVH or LV impairment
Hypertensive heart disease with LVH
Cardiomyopathy (hypertrophic, dilated, or restrictive)
Cardiac sarcoidosis with myocardial involvement
Significant cardiac arrhythmias
Demographic and Other CV Risk Factors:
Age (>65 years)
Family history of premature CV disease
Arterial hypertension
Diabetes mellitus
Hypercholesterolemia
Lifestyle Risk Factors:
Smoking
High alcohol intake
Obesity
Sedentary

Abbreviations: CABG = coronary artery bypass graft; CAD = coronary artery disease; CV = cardiovascular; LV = left ventricular; LVEF = left ventricular ejection fraction; LVH = left ventricular hyperthropy; MI = myocardial infarction; PCI = percutaneous coronary intervention; VHD = valvular heart disease.

Table 2: Cardioprotective Measures for Anthracycline Drugs (adapted from Zamorano et al. 2016)

Potential Cardioprotective Measures for Anthracycline Drugs and Analogues by Limitation of Cumulative Doses	
Drug	**Conversion Factor (Daunorubicin Equivalents)**
Daunorubicin	1
Doxorubicin	2.2
Epirubicin	1.1
Mitoxantrone	5
Idarubicin	5.3

A value of <551 mg/m^2 will be used as the correlative value for liposome-encapsulated annamycin for purposes of assessment of maximal exposure, with a conversion factor of 1.

Consideration of the equivalence with conversion factor and sample calculations of these anthracyclines to calculate prior exposure are provided in Appendix E.

2. STUDY OBJECTIVES

The primary objective of this study is to evaluate the safety and identify the RP2D of liposomal annamycin for the treatment of subjects with AML that is refractory to or relapsed after standard induction therapy.

Secondary objectives:

1. Pharmacokinetics of annamycin and its metabolite, annamycinol
2. Preliminary assessment of the antileukemic activity of liposomal annamycin as second-line therapy for subjects with refractory or relapsed AML based on established response criteria, including complete response (CR), partial response (PR), event-free survival (EFS), overall survival (OS; Kaplan-Meier), and time to and duration of remission/response

3. STUDY DESIGN

3.1 Design Summary

This is a multicenter, open-label, dose-escalation study that will determine the MTD and RP2D of liposomal annamycin as a single agent for the treatment of subjects with AML that is refractory to or relapsed after standard induction therapy.

During the dose-escalation phase of the study, eligible subjects could have received any number of previous therapies for their relapsed AML as long as the prior anthracycline cumulative dose is below the recommended noncardiotoxic level (as defined in Table 2 and Appendix E). During the expansion phase of the study, after the MTD or RP2D has been established, study treatment will be second-line therapy, thus subjects could not have received prior therapy for their relapsed AML.

Enrollment will occur in cohorts of 3 subjects in a conventional 3+3 escalating dose design, starting at a dose level of 100 mg/m^2/day administered for 3 days. Dose escalation will take place on the basis of safety assessments in this first cohort with the next dose of liposomal annamycin escalated to 120 mg/m^2 in the absence of safety concerns.

For each dose level, groups of 3 subjects will receive a treatment cycle of liposomal annamycin daily as a 2-hour IV infusion for 3 consecutive days followed by 18 days off study drug (i.e., the induction treatment cycle = 21 days). If no subject experiences a dose-limiting toxicity (DLT), based on National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) v5, which constitutes a definite study drug-related irreversible or not medically manageable Grade 3 or higher toxicity, further defined in Section 3.4, during the induction treatment cycle through Day 28, the subsequent group of 3 subjects will receive the next higher liposomal annamycin dose. However, if 1 of the 3 initial subjects experiences a DLT, the cohort of subjects at that dose level will be expanded to 6 subjects. If at least 2 of the 6 subjects

experience a DLT, this will be considered a nontolerated dose and then 3 subjects will be treated at a lower dose.

The MTD is defined as the highest dose of liposomal annamycin at which fewer than 2 (of a cohort of up to 6) subjects experience a DLT.

The RP2D is defined as the optimal dose to be determined by the Sponsor in conjunction with the Safety Review Committee/Consultant (SRC), consisting of at least 1 to up to 3 independent consultants, on the basis of review of available clinical and laboratory safety and efficacy data. Up to 21 additional subjects will be enrolled at either the MTD or RP2D to better define toxicity and evaluate efficacy at this dose. Therefore, up to 33 subjects will be enrolled in this study: up to 12 subjects in the dose-escalation phase and up to 21 additional subjects at the MTD or RP2D.

The Medical Monitor and the SRC will continually review all data and decide if the study should continue and if any changes to the protocol before restarting enrollment must be made.

If a subject discontinues treatment for reasons other than study drug-related adverse events such that safety or efficacy of the drug cannot be fully evaluated during the induction cycle, a replacement subject may be enrolled; these circumstances will be reviewed on a case-by-case basis by the SRC in conjunction with the Sponsor.

3.2 Dosing

The initial group of 3 subjects will be treated with a 2-hour intravenous infusion of 100-mg/m^2/day (Dose Level 1) liposomal annamycin daily for 3 consecutive days followed by 18 days off study drug (i.e., 1 treatment cycle = 21 days). Provided that no subject experiences a DLT (defined in Section 3.4) during the induction treatment cycle through Day 28, the ensuing group of 3 subjects will receive 120-mg/m^2/day (Dose Level 2) liposomal annamycin.

The liposomal annamycin dose levels planned for investigation are outlined in Table 3. The starting dose is Dose Level 1.

Table 3: Liposomal Annamycin Dose Levels

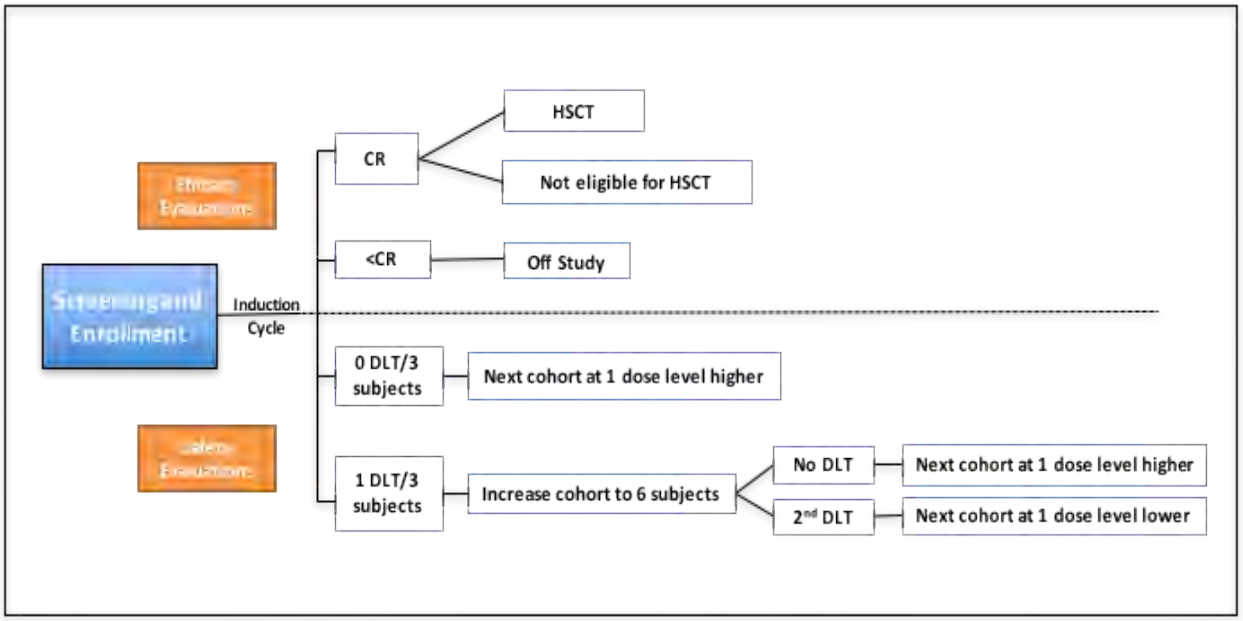
Dose Level Designation	Liposomal Annamycin Dose (mg/m^2/day) × 3 days
-1	90
1	100
2	120

For each dosing cohort, if 1 of the 3 initial subjects experiences a DLT (Section 3.4), the cohort of subjects at that dose level will be expanded to 6 subjects (Figure 1). If at least 2 of the 6 subjects experience a DLT, then 3 subjects will be treated at a lower dose. If a DLT occurs in at least 2 out of 6 subjects at Dose Level 1, the next 3 subjects will be enrolled into Dose Level -1. The MTD is defined as the highest dose of liposomal annamycin at which fewer than 2 (of a cohort of up to 6) subjects experience a DLT. The RP2D is defined as the dose to be

explored in the expansion phase portion of the study, based on the aggregate data of safety and efficacy.

Figure 1: Schematic of Dosing Plan

Liposomal annamycin will be administered for a single induction cycle.



Abbreviations: DLT = dose-limiting toxicity; HSCT = hematopoietic stem cell transplantation.

3.3 Safety Considerations

Subjects will be hospitalized for the full 3 days of liposomal annamycin administration. If a subject experiences a DLT as defined in Section 3.4, or any severe or life-threatening event occurs at any time over the first 3 days, dosing should be stopped and the subject should be permanently discontinued from the study. In addition to the DLT criteria in Section 3.4, the severe or life-threatening events include (but are not limited to) development of an anaphylactic reaction during or after the infusion, clinical or radiological evidence of left ventricular dysfunction with dyspnea and pulmonary rales, cardiomegaly or signs of pulmonary hypertension on chest x-ray, or acute cardiotoxicity (Section 3.3.1).

Additional criteria for safety evaluation after the initial dose during the induction cycle includes creatinine levels increasing to 1.5 times those of baseline or liver function tests increasing to 2 times those of baseline, in which case the next dose of the study drug will be decreased by 25%. If there is further deterioration of organ function after the next dose, the subject will be permanently discontinued from the trial. If these changes are acutely 2 or 3 times those of baseline, respectively, the study drug will be held, and subjects will be monitored for resolution of these changes. If there is prompt resolution (within 2 to 5 days), a rechallenge at a 25% lower dose will be attempted, and if any additional toxicity is observed, the subject will be permanently

discontinued from the trial. If resolution takes longer than 5 days, the subject will not be rechallenged and will be permanently discontinued from the trial.

Subjects successfully completing the first 3 days of dosing will then be evaluated weekly thereafter during the induction cycle of treatment (the induction cycle consists of 21 days [3 weeks total], with the first 3 consecutive days of daily liposomal annamycin treatment followed by 18 days off of liposomal annamycin).

3.3.1 Cardiotoxicity

Subjects will be evaluated before the start of liposomal annamycin treatment and during the first 3 days of liposomal annamycin treatment, with special consideration for potential cardiotoxicity based on documented literature (Section 1.6) that provides currently relevant recommendations. Since acute cardiotoxicity may occur immediately to weeks following treatment and can present as arrhythmias, ST and T wave abnormalities, pericarditis-myocarditis syndrome, and acute heart failure, the subject will be observed in the hospital with daily, diligent attention to evaluation of cardiac status, inasmuch as prompt therapy can provide substantial recovery (Cardinale et al. 2015). Guidelines for limiting the total cumulative dose of anthracyclines will be followed, as per an inclusion criterion that limits any subject with significant cumulative dosing due to prior regimens in addition to anticipated exposure to annamycin (See Section 4 and Appendix E). Subjects will also have clinical and electrocardiographic examinations evaluated at the anticipated maximal plasma concentrations, and again weekly during the induction cycle of treatment with multiple-gated acquisition (MUGA) or echocardiogram (ECHO) scans or ECHO strain evaluations performed every 3 weeks by using the same modality and instrument. Any subject demonstrating any of the above manifestations of cardiotoxicity or >10% decrease in ejection fraction based on scans will be discontinued from the study. Between the Day 18 and Day 28 visits or at the the End of Treatment visit (if the subject is prematurely terminated), a final MUGA or ECHO scan or an ECHO strain evaluation will be conducted by using the same modality and instrument.

3.3.2 Myalgia – Adverse Event Workup

If a subject develops study drug-related ≥Grade 3 myalgia on study, total creatine phosphokinase and creatine phosphokinase isoenzyme measurements should be performed. Appropriate treatment with analgesics should also be initiated.

3.4 Criteria for Dose-Limiting Toxicity

A DLT is defined as any of the following clinically significant AEs or laboratory abnormalities that occur during the DLT evaluation period except those that are clearly and incontrovertibly due to extraneous causes, including disease progression, underlying disease, concurrent illness or concomitant medications. The DLT evaluation period is defined as the first day of treatment (Day 1) until Day 28. DLT observation periods for safety monitoring in expansion cohorts will be as above. The severity of AEs will be graded according to the NCI CTCAE v5.

Any Grade ≥ 3 nonhematologic or extramedullary toxicity with the following exceptions:

- Anorexia or fatigue;
- Grade 3 nausea and/or vomiting if not requiring tube feeding or total parenteral nutrition or diarrhea if not requiring or prolonging hospitalization that can be managed to Grade ≤2 with standard antiemetic or antidiarrheal medications used at prescribed dose within 7 days of onset;
- Grade 3 mucositis that resolves to Grade ≤2 within 7 days of onset;
- Grade 3 fever with neutropenia, with or without infection; or
- Grade 3 infection.

Any subject demonstrating the defining criteria for Hy's Law (Appendix D) will be discontinued from the study, and this will be considered a DLT.

Subjects who do not achieve a remission of leukemia will not be evaluable for hematological toxicity.

3.5 Evaluation of Efficacy

3.5.1 Bone Marrow Evaluation

Bone marrow aspirate (a biopsy if there are no spicules present) 15 to 35 days after the start of therapy will be performed. If cytogenetics were initially abnormal, include cytogenetics as part of the remission documentation.

3.6 Premedications and Prophylactic Treatments

3.6.1 Tumor Lysis Syndrome Prophylaxis

All subjects will receive tumor lysis syndrome prophylaxis as per institutional guidelines or as per published information (Jones et al. 2015; Coiffier et al. 2010).

3.6.2 Allergic Reactions

Antiallergic premedication with an antihistamine (e.g., diphenhydramine at a dose of 50 mg administered intravenously) must be administered before each dose of liposomal annamycin.

Subjects who develop allergic reactions must have their infusions stopped and be treated with corticosteroids and/or diphenhydramine and other supportive measures as deemed appropriate by the Investigator on the basis of institutional standards of care. After the reaction has completely resolved, the infusion can be restarted at 25% of the original rate and slowly increased to 50% of the original rate. For subjects who experience an allergic reaction, prophylaxis with corticosteroids, as well as an antihistamine administered intravenously, will be required for all subsequent infusions. If any subjects experience an anaphylactic reaction to therapy, they will be permanently discontinued from the study.

3.6.3 Oral Prophylaxis for Mucositis

3.6.3.1 Mouthwash

Subjects will receive mouthwash as per the institution's guidelines and practices.

3.6.3.2 Palifermin (Kepivance)

The use of Kepivance should be considered if institutional guidelines and the Investigator recommend it, but is not required. Kepivance is indicated as supportive care for preparative regimens predicted to result in ≥Grade 3 mucositis in the majority of subjects. The recommended dose of Kepivance is 60 µg/kg/day administered as an intravenous bolus injection for 3 consecutive days before and 3 consecutive days after myelotoxic therapy for a total of 6 doses.

3.6.3.3 Oral Cryotherapy

Subjects must receive oral cryotherapy (ice chips) during liposomal annamycin administration, utilize the recommended administration at 5 minutes prior to treatment, and continue for 2 to 6 hours thereafter (Lilleby et al. 2006).

4. ELIGIBILITY CRITERIA

Subjects were diagnosed with AML refractory to or relapsed after standard induction therapy. All subjects must meet the inclusion and exclusion criteria listed below to be eligible for enrollment. Subjects with central nervous system (CNS) involvement will be eligible for this study. They will receive CNS therapy as per standard of care.

4.1 Inclusion Criteria

A subject may be included in this study if he/she meets all of the following criteria:

1. A pathologically confirmed diagnosis of AML by World Health Organization classification

2. AML that is refractory to or relapsed after standard induction therapy

3. Age ≥18 years at the time of signing informed consent

4. No chemotherapy, radiation, or major surgery within 2 weeks prior to first dose of study drug and/or recovered from the toxic side effects of that therapy unless treatment is indicated due to progressive disease

5. No investigational therapy within 4 weeks of the first dose of study drug

6. Eastern Cooperative Oncology Group (ECOG) performance status 0 to 2

7. Adequate laboratory results including the following:
   a. Bilirubin ≤1.5 times the upper limit of normal unless due to Gilbert Syndrome.
   b. Serum glutamic-oxaloacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT), and alkaline phosphatase <3 times the upper limit of normal unless due to organ involvement.
   c. Adequate renal function (The Cockroft-Gault equation will be used to estimate creatinine clearance. This equation is as follows: Creatinine clearance in milliliters per minute = [140 – age] × body weight [kg]/72 × plasma creatinine [mg/dL], multiplied by 0.85 for women. By using this equation, adequate renal function will be deemed to be a creatinine clearance of greater than 60 mL/minute.)

8. Prior anthracycline cumulative dose (see Table 2 and Appendix E) <551 mg/m^2 or the daunorubicin equivalent, which is the recommended noncardiotoxic level. This will be calculated as follows:
   a. Determination of prior exposure will be based on total milligrams per meter squared of prior anthracycline therapy (see Appendix C for commonly used chemotherapy regimens incorporating anthracyclines).
   b. Potential cardioprotective measures for anthracycline drugs and analogues by limitation of cumulative doses are demonstrated by the conversion factors in Table 2.
   c. The maximal allowance will be calculated as a daunorubicin equivalent added to the anticipated exposure to liposomal annamycin with the maximal exposure of anthracyclines capped at 550.9 mg/m^2 (Appendix E).
   d. Exposure to annamycin projected for subjects enrolled in a given cohort in this study will be assessed on the basis of prior total exposure added to the anticipated designated cohort dose level (up to 120 mg/m^2, which, after 3 doses, represents a cumulative exposure of <551-mg/m^2 daunorubicin equivalents).

9. Subject can understand and sign the informed consent document, can communicate with the Investigator, and can understand and comply with the requirements of the protocol.

10. Women of childbearing potential must have a negative serum or urine pregnancy test.

11. All men and women must agree to practice effective contraception during the entire study period and after discontinuing study drug, unless documentation of infertility exists.
   a. Sexually active, fertile women must use 2 effective forms of contraception (abstinence, intrauterine device, oral contraceptive, or double barrier device) from the time of informed consent and until at least 6 months after discontinuing study drug.
   b. Sexually active men and their sexual partners must use effective contraceptive methods from the time of subject informed consent and until at least 3 months after discontinuing study drug.

4.2 Exclusion Criteria

A subject will be excluded from this study if he/she meets any of the following criteria:

1. Subjects diagnosed with acute promyelocytic leukemia

2. Concomitant therapy that includes other chemotherapy that is or may be active against AML, except for prophylaxis and/or treatment of opportunistic or other infection with antibiotics, antifungals, and/or antiviral agents

3. Prior mediastinal radiotherapy

4. Any condition that, in the opinion of the Investigator, places the subject at unacceptable risk if he/she were to participate in the study

5. LVEF <50%, valvular heart disease, or severe hypertension (see Table 1). Cardiac subjects with a New York Heart Association classification of 3 or 4 will be excluded. (Cardiology consultation should be requested if any question arises about cardiac function.) This also includes subjects with baseline QT/QTc interval >480 msec, a history of additional risk factors for torsade des pointes (e.g., heart failure, hypokalemia, family history of Long QT Syndrome), and use of concomitant medications that significantly prolong the QT/QTc interval.

6. Clinically relevant serious comorbid medical conditions including, but not limited to, active infection, recent (less than or equal to 6 months) myocardial infarction, unstable angina, symptomatic congestive heart failure, uncontrolled hypertension, uncontrolled cardiac arrhythmias, chronic obstructive or chronic restrictive pulmonary disease, active CNS disease uncontrolled by standard of care, known positive status for human immunodeficiency virus and/or active hepatitis B or C, cirrhosis, or psychiatric illness/social situations that would limit compliance with study requirements

7. Pregnant, lactating, or not using adequate contraception

8. Known allergy to anthracyclines

9. Any evidence of mucositis/stomatitis or previous history of severe (≥Grade 3) mucositis from prior therapy

10. Required use of strong inhibitors and inducers of the Cytochrome P450 family of enzymes (CYP) and transporters that cannot be held during treatment days

4.3 Subject Withdrawal

Subjects may voluntarily withdraw from the study at any time for any reason and without prejudice to further treatment or the Investigator may withdraw subjects if necessary.

Subjects who are removed from the study will undergo all study evaluations described for the End of Study visit (Section 6.4). The reasons for withdrawal will be recorded.

The reasons for which a subject may be prematurely discontinued include the following:

1. Subject has unacceptable toxicity, subject demonstrates manifestations of cardiotoxicity, or subject has >10% decrease in ejection fraction based on scans. All subjects who are discontinued as a result of an adverse event should be followed for as long as necessary to document the resolution or stabilization of the event.

2. Subjects with disease progression

3. Subject withdrawal of consent

4. Investigator's discretion

5. General or specific changes in the subject's condition that renders the subject ineligible for further treatment

6. If pregnancy occurs or is suspected during study drug treatment, annamycin must be discontinued immediately. The subject should be referred to an obstetrician/gynecologist experienced in reproductive toxicity for further evaluation and counseling. If none is immediately available, contact Moleculin Biotech for a referral.

7. If the subject's continued participation poses unacceptable risk for any other reason or if they are not compliant with the study

4.3.1 Withdrawal Procedures

Subjects withdrawn from the study will complete the end of study (EOS) procedures described in Section 6.4 and be followed until resolution of any AEs or until the unresolved AEs are judged by the Investigator to have stabilized.

The Investigator will promptly notify the Medical Monitor of a subject’s withdrawal for reasons related to toxicity.

4.3.2 Subject Replacement

Subjects withdrawing in the cohort dose-escalation period for reasons other than toxicity who have not taken all 3 doses during the induction cycle and/or fail to complete restaging after completion of the treatment cycle such that safety is not evaluable will be replaced. However, subjects experiencing a DLT in the dose-finding cycle will not be replaced.

5. STUDY TREATMENT

5.1 Liposomal Annamycin (Study Drug)

5.1.1 Description of Liposomal Annamycin

Annamycin is a lipophilic anthracycline antibiotic that incorporates 4 structural modifications from doxorubicin: 2'-iodo, 3'-hydroxy, 4'-epi, 4-demethoxy doxorubicin. Annamycin is completely insoluble in water but soluble in chloroform and dimethyl sulfoxide.

Liposomal annamycin will be supplied by Moleculin Biotech, Inc., in 50-mL vials containing annamycin as a lyophilized powder. The study drug also contains phospholipids (dimyristoylphosphatidyl choline and dimyristoylphosphatidyl glycerol) and polysorbate 20.

Study drug will be shipped to the pharmacy at the study site in individually labeled vials. Each vial is labeled with the following information: drug name, strength, lot number, date of manufacture, storage conditions, sponsor's address, manufacturer's name, and the statement "Caution: New Drug - Limited to Investigational Use."

This study is an open-label study, and study drug will not be blinded, nor will placebo product be administered.

5.1.2 Preparation of Liposomal Annamycin

The study drug must be reconstituted prior to use. The diluted product can be held for up to 24 hours when stored between 34 to 42ºC, after which time unused drug should not be administered to subjects.

Caution should be exercised in the handling and preparation of liposomal annamycin. The use of gloves is required. If liposomal annamycin comes into contact with skin or mucosa, immediately wash thoroughly with soap and water. Liposomal annamycin should be handled in a manner consistent with other anticancer drugs.

5.1.3 Liposomal Annamycin Administration

Subjects will be treated daily for 3 consecutive days with a 2-hour intravenous infusion of liposomal annamycin followed by 18 days off liposomal annamycin (i.e., 1 treatment cycle = 21 days). A peripheral vein may be used. The starting dose of liposomal annamycin (dose for subjects in Cohort 1) is 100 mg/m^2/day. The liposomal annamycin doses will be escalated in sequential cohorts. Discussions of dose escalation and modifications are provided in Section 3.2 and Table 3.

5.1.4 Storage

At the study site, all investigational study drugs will be stored in a locked, safe area to prevent unauthorized access. The lyophilized liposomal annamycin powder should be stored in the

freezer at -10 to -25°C (-13 to 14°F). The reconstituted liposomal annamycin suspension is prepared as described in the Pharmacy Manual.

5.2 Concomitant Medications

All medications, whether prescription or nonprescription and including pharmacologic doses of vitamins, must be recorded on the Electronic Case Report Form/Electronic Data Capture (eCRF/EDC).

Subjects must not be receiving chemotherapeutics that are or may be active against AML, including investigational agents, while taking study drug, except for prophylaxis and/or treatment of opportunistic or other infection with antibiotics, antifungals, and/or antiviral agents. Subjects should not be undergoing concurrent radiation therapy. No new investigational treatments are to be initiated during the subject's participation in the study.

On the basis of the known metabolism of anthracyclines by CYP3A and CYP2B, enzymes of the Cytochrome P450 family, strong inducers and inhibitors of these enzymes will be prohibited during the days of treatment. Information about relevant drugs can be accessed through this website: http://bioinformatics.charite.de/transformer/, which easily provides drug interaction information; however, any concerns should be discussed with the Sponsor.

5.3 Measuring Subject Compliance

Study drug will be administered only at the investigational site and to subjects enrolled in this clinical study under the supervision of the Investigator in accordance with the protocol and institutional practice. Administration of drug will be recorded on the eCRF/EDC. Under no circumstances is the Investigator allowed to release clinical supplies for use by another physician not named on Form Food and Drug Administration (FDA) 1572, or to administer assigned study drugs to a subject who is not enrolled in this study. Study drug will be dispensed only at the institution specified on Form FDA 1572 for US sites. For EU sites, FDA form will only be collected if permitted by the management of the participating institutions or local/national guidelines and regulations.

5.4 Drug Accountability

In accordance with current Good Clinical Practice (GCP), the investigational site will account for all supplies of annamycin. Details of receipt, storage, administration, and return or destruction will be recorded in the study drug accountability record according to the standard operating procedures of the investigational site. Copies of the study drug accountability record will be provided to the Sponsor.

At the end of the study, Moleculin Biotech, Inc., will instruct the Investigator on the return or destruction of unused medication. If any medication is lost or damaged, its disposition should be documented. Investigational supplies will be retained at the clinical site pending instructions for disposition by Moleculin Biotech, Inc., at the end of the study.

6. STUDY PROCEDURES

See Appendix A and Appendix B.

6.1 Screening

The Investigator is responsible for keeping a record of all subjects screened for entry into the study and subsequently excluded. The reason(s) for exclusion must also be recorded. Written informed consent must be given before any study related diagnostic or screening procedures, unless performed as part of standard of care. The following screening procedures must be performed within 35 days prior to study entry:

1. Informed consent
2. Refractory or relapsed AML diagnosis confirmed
3. ECOG performance status (Appendix B)
4. Complete medical history including documentation of all treatments given for AML, cytogenetic and molecular abnormalities obtained at diagnosis, as well as a full cardiac history including assessment of prior anthracycline exposure and calculation of potential additional exposure based on assignment to dosing cohort (Section 4)
5. Physical examination including height, weight, vital signs, and oral examination
6. Electrocardiogram (ECG)
7. Blood chemistry (sodium, potassium, chloride, bicarbonate, calcium, magnesium, phosphorus, blood urea nitrogen [BUN], creatinine, glucose, albumin, total protein, alkaline phosphatase, total bilirubin, SGOT, SGPT], lactate dehydrogenase [LDH], and uric acid) with creatinine clearance to be calculated from these results
8. Complete blood count (CBC) with differential and platelet count
9. Urinalysis
10. Concomitant medications used within 30 days prior to the first dose of study drug
11. Pregnancy test (with sensitivity of at least 50 mIU/mL) for women of childbearing potential within 72 hours prior to first dose of study drug
12. MUGA or ECHO scan or ECHO strain evaluation within 2 weeks prior to first dose of study drug

6.2 Baseline (Day 1)

The subject will be seen at the scheduled baseline visit (Day 1). The Investigator will certify that the subject continues to satisfy all inclusion and exclusion criteria. If eligible, the subject will be enrolled into the study and will be assigned an identification number in sequential order of enrollment. The following baseline evaluations will be made just prior to dosing on Day 1 (unless otherwise indicated):

1. Updated medical history
2. Physical examination including weight, vital signs, and oral examination
3. ECOG performance status (Appendix B)
4. Blood samples for pharmacokinetic analysis and cardiac biomarkers (troponin-I and troponin-T) collected just prior to the start of the annamycin infusion on Day 1
5. Bone marrow aspirate and peripheral blood (complete blood count, differential, and platelet count; flow cytometry is optional) within 15 days prior to the first dose of liposomal annamycin to document disease
6. Updated concomitant medications

If screening was ≥14 days prior to first dose of study drug, the following should be repeated:

1. Blood chemistry (sodium, potassium, chloride, bicarbonate, calcium, magnesium, phosphorus, BUN, creatinine, glucose, albumin, total protein, alkaline phosphatase, total bilirubin, SGOT, SGPT, LDH, and uric acid) with creatinine clearance to be calculated from these results
2. CBC with differential and platelet count
3. Urinalysis
4. ECG

6.3 On-Study Evaluations

6.3.1 Study Drug Administration

Dosing will be in the morning at approximately the same time each day. Subjects will be treated with a daily 2-hour intravenous infusion of liposomal annamycin for 3 consecutive days. Prophylaxis as described in Section 3.6 will be administered. Antiallergic premedication with diphenhydramine at a dose of 50 mg administered intravenously must be administered before each dose of liposomal annamycin. During each period of study drug administration subjects must be observed for at least 4 to 6 hours following daily liposomal annamycin administration. The following evaluations will be conducted during the 3 days of liposomal annamycin administration (Days 1 to 3):

1. Physical examination including weight, vital signs, and oral examination on Days 2 and 3

2. Blood samples for pharmacokinetic analysis collected at predose and at 0.25, 0.5, 1, 2, 4, 8, and 24 hours after the start of liposomal annamycin infusion on Day 1 and Day 3 for 3 subjects at each dose and 6 subjects at the RP2D who complete the 3 days of dosing

3. Blood samples for cardiac biomarkers (troponin-I and troponin-T) collected at predose, at the end of infusion on Day 3, and on Day 21

4. ECG monitoring at predose and at 4 hours and 24 hours after the start of liposomal annamycin infusion on Day 1, Day 2, and Day 3 for all subjects at each dose and 6 subjects on Day 1 and Day 3 only at the RP2D who complete the 3 days of dosing, coinciding with pharmacokinetic (PK) evaluations

5. Monitoring of adverse events and review of concomitant medications and concurrent illnesses (treatment-emergent or worsening illness must be recorded as an adverse event). Evaluation of blood chemistries and blood counts daily. Over the 3 days of initial dosing, if any toxicity occurs, including blood count abnormalities, dosing should be interrupted until resolution, with rechallenge at a 25% lower dose, based on guidelines in Sections 3.3 and 3.4. If any DLT is encountered (as defined in Sections 3.3 and 3.4) including any severe or life-threatening event, the subject should be discontinued from the study. These specific events include (but are not limited to) development of an anaphylactic reaction during or after the infusion or clinical or radiological evidence of left ventricular dysfunction with dyspnea and pulmonary rales, cardiomegaly, or signs of pulmonary hypertension on chest x-ray.

6. Concomitant medications

6.3.2 Induction Cycle

Subjects will return to the clinic weekly on Days 7, 14, and 21 during the induction cycle.

The evaluations listed below will be conducted at each weekly evaluation during the induction cycle (Appendix A):

1. Monitoring of adverse events and review of concurrent illnesses (treatment-emergent or worsening illness must be recorded as an adverse event)

2. Concomitant medications

3. Physical examination including weight, vital signs, and oral examination

4. CBC with differential and platelet count

5. Blood chemistry (sodium, potassium, chloride, bicarbonate, calcium, magnesium, phosphorus, BUN, creatinine, glucose, albumin, total protein, alkaline phosphatase, total bilirubin, SGOT, SGPT, LDH, and uric acid)

6. Pregnancy test (with sensitivity of at least 50 mIU/mL) for women of childbearing potential on Day 7

7. ECG

In addition to the weekly evaluations during the induction cycle, the following evaluations will be conducted at the end of the 3-week treatment cycle:

1. MUGA or ECHO scan or ECHO strain evaluation between Days 18 and 28. Cardiology consultation should be requested if any question arises about cardiac function. The MUGA scan should also be conducted at the Early Termination visit for subjects who are withdrawn prematurely.

2. Bone marrow aspirate and peripheral blood specimens (complete blood count, differential, and platelet count; flow cytometry is optional) between Days 15 and 35 to document response

3. Pregnancy test (with sensitivity of at least 50 mIU/mL) for women of childbearing potential on Day 21

4. Blood sample for cardiac biomarkers on Day 21

6.4 End of Study Evaluations

The subject will return to the clinic for an EOS evaluation within 1 week of their treatment cycle or last study drug administration if the treatment period was prematurely terminated or subject was withdrawn. In addition, any subject with a treatment-related medical event that requires additional follow-up must continue to be monitored as medically appropriate until resolution or stabilization (in the event that resolution is not possible). The procedures listed below are to be undertaken at the EOS visit:

1. ECOG performance status (Appendix B)

2. Blood chemistry (sodium, potassium, chloride, bicarbonate, calcium, magnesium, phosphorus, BUN, creatinine, glucose, albumin, total protein, alkaline phosphatase, total bilirubin, SGOT, SGPT, LDH, and uric acid)

3. CBC with differential and platelet count

4. Monitoring of adverse events and review of concurrent illnesses (treatment-emergent or worsening illness must be recorded as an adverse event)

5. Concomitant medications

6.5 Follow-up Evaluations

Follow-up evaluations will occur 2 weeks after the EOS visit. The procedures listed below are to be undertaken at the Follow-up visit:

1. Physical examination including weight, vital signs, and oral examination

2. Monitoring of adverse events and review of concurrent illnesses (treatment-emergent or worsening illnesses must be recorded as an adverse event). Any subject with a treatment related medical event must continue to be monitored as medically appropriate until resolution or stabilization (in the event that resolution is not possible).

3. Concomitant medications

6.6 Survival Follow-up

Subjects will be followed every 3 months from study drug discontinuation to monitor resolution or stabilization of any treatment-related medical events and obtain survival data. If possible, a MUGA scan, ECHO scan, or ECHO strain evaluation will be collected.

7. ASSESSMENT OF EFFICACY

7.1 Efficacy Parameters

7.1.1 Response Criteria

All subjects evaluable for efficacy will be assessed for response to treatment by using the recommendations of the International Working Group for standardization of response criteria, treatment outcomes, and reporting for therapeutic trials (Cheson et al. 2003). The primary efficacy variable is leukemia response rate, evaluated by the Investigator at the end of the cycle and at the EOS visit, based on bone marrow aspirate and peripheral blood collected at the end of the treatment cycle. A bone marrow (BM) aspirate (biopsy if there are no spicules present) should be repeated in 1 week if there is a question of residual leukemia in assessing efficacy based on an initial BM specimen.

An evaluation of response to therapy is one of the objectives of this study. Response criteria are described below:

Primary Endpoints:

1. Morphologic leukemia-free state
   a. BM <5% blasts in an aspirate with spicules (a BM biopsy should be performed if spicules are absent)
   b. No blasts with Auer rods or persistence of extramedullary disease
2. Complete remission:
   a. Morphologic leukemia-free state in which
      i. Subject is independent of transfusions
      ii. Absolute neutrophil count of >1,000
      iii. Platelets of ≥100,000
3. Cytogenetic complete response – cytogenetics normal in those with previously abnormal cytogenetics
4. Cri and CRp: CR with incomplete blood count recovery meets all criteria for CR except for either neutropenia (<1,000; CRi) or thrombocytopenia (<100,000; CRp) but must include transfusion independence

Secondary Endpoints:

1. EFS: Time from enrollment until disease progression or death from any cause
2. OS: Time from enrollment until death from any cause
3. PR: All of the hematologic values for a CR, with normalization of blood counts, but with a decrease of at least 50% in the percentage of blasts to 5% to 25% in the bone marrow aspirate
4. Time to and duration of remission/response

7.2 Methods for Measuring Efficacy

Bone marrow aspirate/biopsy and peripheral blood specimens (complete blood count, differential, and platelet count) to document response will be collected at 15 to 35 days after the start of therapy. If cytogenetics were initially abnormal, include cytogenetics as part of the remission documentation. After documentation of remission, these procedures should be performed every cycle thereafter. In addition, flow cytometry analysis of peripheral blood may be utilized for response assessment.

8. ASSESSMENT OF SAFETY

8.1 Safety Parameters

A primary objective of this study is to evaluate the safety and identify the MTD of liposomal annamycin when given in doses starting at 100 mg/m^2/day and ranging to as high as 120 mg/m^2/day, or the MTD, whichever is lower, in subjects with refractory or relapsed AML. The Investigators, meeting on a regular basis, will discuss any safety issues arising in the study in conjunction with the Sponsor and the medical monitor. The designation of an SRC (Section 8.5) will be available to adjudicate any issues concerning safety, attribution, or subject disposition.

8.2 Methods for Measuring Safety

Clinical AEs, routine hematological and biochemical parameters, physical examination and vital signs, 12-lead ECG, ECOG performance status, pregnancy testing, and monitoring of concomitant medications will be assessed according to the Schedule of Events (Appendix A).

All AEs and laboratory abnormalities will be graded for severity by using the NCI CTCAE v5. The relatedness of all AEs and laboratory abnormalities and their seriousness will be determined by the Investigator and adjudicated by the SRC in conjunction with the Sponsor. The SRC will consist of at least 1 and up to 3 external, independent (not participating in the clinical trial) consultants, providing guidance with regard to safety issues, including approval of dose escalation and designation of DLTs.

AEs will be documented from the time of first dose of study drug until 39 days after the last dose administered on study.

Strategies for detection of cardiotoxicity that include cardiac imaging (echocardiography, nuclear imaging, cardiac magnetic resonance) and biomarkers (troponin, natriuretic peptides) will be based on clinical assessments at screening, as well as current relevant guidelines (Zamorano et al. 2016; Cardinale et al. 2015).

8.3 Procedures for Recording Adverse Events

All adverse drug events encountered during the clinical study will be recorded on the eCRF/EDC. Adverse drug events are defined in the International Conference on Harmonisation (ICH) Guidance for Industry E6: Good Clinical Practice as follows:

> An AE is any untoward medical occurrence in a subject or clinical investigation subject administered a pharmaceutical product and that does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (ICH April 1996).

An adverse event is any adverse change from the subject's baseline condition, including any clinical or clinically significant laboratory test value abnormality that occurs during the course of the clinical study after the experimental drug has been utilized, whether the adverse event is considered related to the treatment or not.

All adverse events will be followed until resolution. This may require obtaining clinical blood samples for appropriate laboratory tests until their values return to baseline levels or performing end of study or follow-up physical examinations until resolution of identified abnormalities.

8.3.1 Routine Reporting of Adverse Events

Adverse events, whether or not associated with study drug administration, will be recorded on the Adverse Event Report form of the eCRF/EDC and will be submitted to the Sponsor at regularly scheduled intervals.

The information to be entered in the eCRF/EDC will include:

1. The time of onset of any new adverse event or the worsening of a previously observed adverse event

2. The specific type of reaction in standard medical terminology

3. The duration of the adverse event (start and stop dates)

4. The severity/grade of the adverse event. The severity should be rated according to the NCI CTCAE v5.

5. An assessment should be made of the relationship of the adverse event to the study drug, i.e., according to the definitions in Section 8.3.4.

6. Description of action taken in treating the adverse event and/or change in study drug administration or dose

7. Outcome

Follow-up assessments should be repeated to document return of any abnormalities to normal or to document other outcome of the adverse event.

8.3.2 Reporting of Serious Adverse Drug Events, Including Deaths

A serious adverse event (SAE), as defined in 21 Code of Federal Regulations 312.32 is:

Any adverse drug event occurring at any dose that results in any of the following outcomes:

1. Death

2. A life-threatening adverse drug event (i.e., immediate risk of death from the event as it occurred; this does not include an adverse event that, had it occurred in a more serious form, might have caused death)

3. Inpatient hospitalization or prolongation of existing hospitalization

4. A persistent or significant disability/incapacity

5. Congenital anomaly/birth defect

Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug event when, based upon appropriate medical judgment, they may jeopardize the subject or subject and may require medical or surgical intervention to prevent 1 of the outcomes listed in this definition. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Serious adverse events, including death due to any cause, which occur from the time subject has taken the first dose of study drug or within 30 days following the last dose of study medication, regardless of study drug relation to the SAE, or any SAE occurring later than 30 days following the last dose of study medication with Investigator opinion as related to the study medication, must be reported immediately to Theradex Safety Desk (within 24 hours of the site becoming aware of the event) by email, fax, or telephone. Notification by email is preferred. The fax and telephone numbers and the email address listed below may be used during both business and nonbusiness hours. During nonbusiness hours a recorded message will provide the telephone caller with the contact information for the on-call monitor.

All SAEs require that a serious adverse event report form be completed and forwarded either via facsimile or as a PDF via email to Theradex® at the fax number or email listed below within 24 hours of the site becoming aware of the event.

All SAEs will be reported to:	Theradex Safety Desk
Email:	SafetyDeskUS@theradex.com
Fax:	1-609-799-1567
Telephone:	1-609-799-7580

In addition to the reporting of SAEs to the Theradex Safety Desk, the SAE must be reported to the Independent Ethics Committee/Institutional Review Board (IEC/IRB) according to their requirements, as well as to regulatory agencies based upon local reporting requirements.

If the SAE is not previously documented in the Investigator's Brochure and is thought to be related to the investigational treatment the Sponsor may urgently require futher information from the Investigator for Health Authority reporting. The Sponsor may need to issue an Investigator Notification to inform all Investigators involved in any study with the same investigational treatment that this SAE has been reported.

Hospitalizations due to the underlying disease will not be reported as an SAE unless there is reason to suspect a causal relationship with the study drug.

8.3.3 Reporting of Suspected Unexpected Serious Adverse Reactions

Suspected Unexpected Serious Adverse Reactions will be collected and reported to the competent authorities and relevant ethics committees in accordance with EU Guidance 2001/C172/01 or as per national regulatory requirements in participating countries.

8.3.4 Severity of Adverse Drug Events

Adverse drug events will be graded according to the criteria in the NCI CTCAE v5. The toxicity criteria can be accessed through the NCI website (https://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm).

8.3.5 Relationship of Adverse Events to the Study Drug

The Investigator should record on the eCRF/EDC whether the relationship of the adverse event is best described as unrelated, possibly related, probably related, or related to the study medication, according to the following definitions.

> **Unrelated:** There is evidence that the adverse event definitely has an etiology other than the assigned study drug.
>
> **Possibly Related:** The adverse event has a temporal relationship to study drug administration. However, an alternative etiology may be responsible for the adverse event. Information on drug/product withdrawal may be lacking or unclear.
>
> **Probably Related:** The adverse event has a temporal relationship to study drug administration. The event is unlikely to be related to an alternative etiology. There is a

reasonable response on withdrawal (dechallenge). Rechallenge information is not required.

Related: The adverse event has a temporal relationship to study drug administration and resolves when the drug is discontinued. An alternative etiology is not apparent. If the subject is rechallenged with the assigned study drug, the adverse event recurs. Rechallenge is not necessarily required.

8.4 Withdrawal From Study Treatment Due to Adverse Events

Subjects withdrawn from receiving additional study drug as a result of an adverse event will be followed by the Investigator until the outcome is determined. Additional reports will be provided to the Sponsor or regulatory authorities when requested. Every effort will be made to follow the subject for the full study period as per the Schedule of Events (Appendix A).

8.5 Establishment of a Safety Review Committee/Consultant

At least 1 and up to 3 clinicians with the relevant expertise in AML, as well as experience in clinical trials, who are not participating in the current clinical study, will be assigned as the SRC to review safety considerations that affect dosing and drug administration. Regular as well as well as ad hoc meetings will be convened to ensure subject disposition is based on an independent review of data and assessment of risk/benefit for subjects. Designation of the appropriate individual(s) will be based on review and approval by the responsible IRBs.

8.6 Monitoring of Cardiovascular Toxicity

Blood samples will be drawn to assess cardiac biomarkers (troponin-I and troponin-T) at predose, at the end of the infusion on Day 3, and on Day 21.

9. PHARMACOKINETIC ASSESSMENT

The pharmacokinetics of annamycin and its metabolite, annamycinol will be determined. Blood samples for pharmacokinetic analysis collected at predose and at 0.25, 0.5, 1, 2, 4, 8, and 24 hours after the start of liposomal annamycin infusion on Day 1 and Day 3 for 3 subjects at each dose and 6 subjects at the RP2D who complete the 3 days of dosing.

ECGs and documentation of concomitant medications affecting CYP enzymes will be obtained to ensure that these are a consideration in the evaluation of the safety and pharmacokinetics of this drug. ECG determinations for subjects at the RP2D will only be required on Days 1 and 3, coinciding with PK assessments.

Instructions for the collection, processing, and shipment of samples will be provided in the Pharmacokinetic Manual.

Plasma levels of annamycin and annamycinol will be determined by validated liquid chromatography with tandem mass spectroscopy. Concentration-time profiles of annamycin and

annamycinol will be analyzed by noncompartmental methods and/or nonlinear least squares regression by using WinNonlin (Pharsight Corporation). Pharmacokinetic parameters and variables will be calculated according to standard equations.

10. STATISTICS

This is a multicenter, open-label, dose-escalation, MTD/RP2D safety study of liposomal annamycin given to subjects with refractory or relapsed AML. Therefore only descriptive statistics will be utilized. A maximum of 33 subjects will be recruited for the study depending on the level at which toxicity is observed.

Data from all subjects who receive 1 or more doses of liposomal annamycin will be incorporated into the final safety analysis. Safety and tolerability will be assessed by adverse events, vital signs, physical examinations, laboratory parameters, and MUGA or ECHO scans or ECHO strain evaluations, as well as cardiotoxicity biomarkers. Adverse events will be listed by subject, and will be tabulated and summarized as the number and percentage of subjects who reported each adverse event. Descriptive statistics will be generated as appropriate; otherwise, no formal statistical analyses are planned on the safety data.

Pharmacokinetic parameters (maximum concentration, area under the time-concentration curve , elimination half-life, volume of distribution, and clearance) will be calculated for annamycin and its metabolite, annamycinol, on the basis of plasma concentrations. Pharmacokinetic data will be analyzed by descriptive statistics as appropriate.

ECGs and documentation of concomitant medications affecting CYP enzymes will be obtained to ensure that these are a consideration in the evaluation of the safety and pharmacokinetics of this drug.

The Medical Monitor and the SRC will continually review all data and decide if the study should continue and if any changes to the protocol before restarting enrollment must be made.

11. ACCESS TO SOURCE DOCUMENTS

The Investigator will make the source documents for this trial available to the sponsor or its representatives, or to the regulatory authority or health authority inspectors.

Subject medical information obtained as a result of this study is considered confidential and disclosure to third parties other than those noted below is prohibited. All reports and communications relating to subjects in this study will identify each subject only by their initials and number. Medical information resulting from a subject's participation in this study may be given to the subject's personal physician or to the appropriate medical personnel responsible for the subject's welfare. Data generated as a result of this study are to be available for inspection on request by FDA or other government regulatory agency auditors, the Sponsor Clinical Monitor (or designee), and the IRB/IEC.

The information developed in this clinical study will be used by the Sponsor in the clinical development of the study medication and therefore may be disclosed by the Sponsor as required for disclosure as a public company to other clinical investigators, to other pharmaceutical companies, to the FDA, and to other government agencies.

Any information, inventions, or discoveries (whether patentable or not), innovations, suggestions, ideas, and reports, made or developed by the Investigator(s) as a result of conducting this study shall be promptly disclosed to the Sponsor and shall be the sole property of the Sponsor. The Investigator agrees upon the Sponsor's request and at the Sponsor's expense to execute such documents and to take such other actions as the Sponsor deems necessary or appropriate to obtain patents in the Sponsor's name covering any of the foregoing.

The Investigator shall retain the clinical study records until such time as directed by Moleculin. At a minimum, records are to be retained for at least 15 years after completion of the study. The Investigator will notify the sponsor prior to destroying any study records. Should the investigator wish to assign the study records to another party or move them to another location, the sponsor must be notified in writing in advance.

If the Investigator cannot guarantee this archiving requirement at the study site for any or all of the documents, special arrangements will be made between the Investigator and the sponsor for storage. If source documents are required for continued care of the subject, appropriate copies for storage off site will be made.

12. QUALITY CONTROL AND QUALITY ASSURANCE

Study monitors will periodically audit, at mutually convenient times during and after the study, all eCRF/EDCs and corresponding office and clinical laboratory records for each subject. The monitoring visits provide the sponsor with the opportunity to evaluate the progress of the study, to verify the accuracy and completeness of eCRF/EDCs, to resolve any inconsistencies in the study records, and to assure that all protocol requirements, applicable Health Authority regulations, other requirements, and Investigator's obligations are being fulfilled.

13. ETHICS

13.1 Declaration of Helsinki

The study will be conducted in accordance with the Declaration of Helsinki (1964) including all amendments up to and including the Fortaleza, Brazil revision (2013) as described in "Ethical Principles for Medical Research Involving Human Subjects," which can be accessed at http://www.wma.net/en/30publications/10policies/b3/.

13.2 Institutional Review Board

The protocol, informed consent form, and any materials (such as advertisements, subject information sheets, or descriptions of the study used to obtain informed consent) for this study will be reviewed and approved by a duly constituted IRB/IEC.

The Investigator will ensure that all aspects of the IRB/IEC review are conducted in accordance with current institutional, local, and national regulations. A letter documenting the IRB/IEC approval and, in Europe, also the national Competent Authority approval will be provided to the sponsor prior to initiation of the study. Amendments to the protocol will be subject to the same requirements as the original protocol. The Investigator or, in Europe, the Sponsor's representative will submit all periodic reports and updates that the IRB/IEC may require, including any final close out reports. The Investigator or, in Europe, the Sponsor's representative will inform the IRB/IEC of any reportable adverse events.

13.3 Informed Consent

Each subject will be provided with oral and written information describing the nature and duration of the study in a language they can understand and must consent in writing to participate before undergoing screening. The date of the consent shall be entered by the subject. The original signed consent form will be retained with the study center's records. Each subject will also be given a copy of his/her signed consent form.

14. DATA HANDLING AND RECORDING

eCRF/EDCs must be completed for every subject who signs an informed consent and has received study drug.

The Investigator is responsible for the completeness and accuracy of information collected on the eCRF/EDCs for each individual enrolled. The eCRF/EDCs will be signed and dated by the Investigator.

The sponsor or designee will conduct data processing. Completed eCRF/EDCs will be reviewed carefully for accuracy and completeness. If necessary, the study site will be contacted for corrections and/or clarifications. All data will be entered into a study database for analysis and reporting. Any data captured electronically (such as laboratory data) will be transferred electronically to the database. Upon completion of data entry, the database will receive a quality assurance check to ensure acceptable accuracy and completeness.

15. PUBLICATION POLICY

The result of this study will be published under the direction of the Sponsor. Results will not be published without prior review and approval by the Sponsor.

16. PROTOCOL AMENDMENTS AND MODIFICATIONS

The Investigator will ensure that the study is conducted in accordance with the procedures and evaluations described in this protocol. The Sponsor is responsible for all protocol amendments and modifications, except those intended to reduce immediate risk to subjects. The Sponsor is responsible for submitting protocol amendments to the appropriate government regulatory authorities. In the USA, the Investigator is responsible for submitting protocol amendments to

the appropriate IRB/IEC. Approval by the IRB will be obtained before changes are implemented unless the Investigator concludes that an emergency exists. In Europe, the Sponsor representative will obtain Competent Authority and IEC approval on behalf of the participating sites. Other local/national approvals may be required depending on the country.

When an emergency occurs that requires a departure from the protocol for a subject, a departure will be only for that subject. The Investigator or other physician in attendance in such an emergency will, if circumstances and time permit, contact Moleculin Biotech, Inc., immediately by telephone. Such contacts will be made as soon as possible to permit a decision as to whether or not the subject is to continue in the study. The departure from the protocol will be described completely, including the reasons for the departure, in the subject's eCRF/EDC. In addition, the IRB will be notified in writing of such departure from protocol.

17. REFERENCES

Acton EM, Tong GL, Mosher CW, Wolgemuth RL. Intensely potent morpholinyl anthracyclines. J Med Chem. 1984;27(5):638-45.

Andreeff M, Giles F, Kornblau S, Konopleva M., Leysath C, Jackson C, et al. Phase I study of annamycin, a novel anthracycline, in patients with relapsed/refractory acute myeloid and lymphoid leukemias [abstract]. Proc Am Soc Clin Oncol. 2001;20:303a. Abstract 1211.

Barbieri B, Giuliani FC, Bordoni T, Casazza AM, Geroni C, Bellini O, et al. Chemical and biological characterization of 4'-iodo-4'-deoxydoxorubicin. Cancer Res. 1987;47(15):4001-6.

Booser DJ, Esteva FJ, Rivera E, Valero V, Esparza-Guerra L, Priebe W, Hortobagyi GN. Phase II study of liposomal annamycin in the treatment of doxorubicin-resistant breast cancer. Cancer Chemother Pharmacol. 2002;50(1):6-8.

Booser DJ, Perez-Soler R, Cossum P, Esparza-Guerra L, Wu QP, Zou Y, et al. Phase I study of liposomal annamycin. Cancer Chemother Pharmacol. 2000;46(5):427-32.

Cancer Stat Facts: Leukemia - Acute Myeloid Leukemia (AML) [Internet]. Bethesda: National Cancer Institute Division of Cancer Control and Population Sciences; [cited 20 Sep 2016]. Available from: https://seer.cancer.gov/statfacts/html/amyl.html.

Cardinale D, Colombo A, Bacchiani G, Tedeschi I, Meroni CA, Veglia F, et al. Early detection of anthracycline cardiotoxicity and improvement with heart failure therapy. Circulation. 2015;131(22):1981-8.

Chen X, Xie H, Wood BL, Walter RB, Pagel JM, Becker PS, et al. Relation of clinical response and minimal residual disease and their prognostic impact on outcome in acute myeloid leukemia. J Clin Oncol. 2015;33(11):1258-64.

Cheson BD, Bennett JM, Kopecky KJ, Büchner T, Willman CL, Estey EH, et al. Revised recommendations of the International Working Group for diagnosis, standardization of response criteria, treatment outcomes, and reporting standards for therapeutic trials in acute myeloid leukemia. J Clin Oncol. 2003;21(24):4642-9.

Coiffier B, Altman A, Pui CH, Younes A, Cairo MS. Guidelines for the management of pediatric and adult tumor lysis syndrome: an evidence-based review. J Clin Oncol. 2008;26:2767-78. Erratum in: J Clin Oncol. 2010;28(4):708.

Consoli U, Priebe W, Ling YH, Mahadevia R, Griffin M, Zhao S, et al. The novel anthracycline annamycin is not affected by P-glycoprotein-related multidrug resistance: comparison with idarubicin and doxorubicin in HL-60 leukemia cell lines. Blood. 1996;88(2):633-44.

Dalton WS. Multidrug resistance. In: DeVita V, Hellman S, Rosenberg S, editors. Cancer principles and practice of oncology. Fourth ed. Philadelphia: J.B. Lippincott Co.; 1993.

Deschler B, Lübbert M. Acute myeloid leukemia: epidemiology and etiology. Cancer. 2006;107(9):2099-107.

DeVita V, Hellman S, Rosenberg S, eds. Cancer principles and practice of oncology. Fourth ed. Philadelphia: J.B. Lippincott Co.; 1993.

Ferrara F, Schiffer CA. Acute myeloid leukaemia in adults. Lancet. 2013;381(9865):484-95.

Gabizon A, Dagan A, Goren D, Barenholz Y, Fuks Z. Liposomes as in vivo carriers of adriamycin: reduced cardiac uptake and preserved antitumor activity in mice. Cancer Res. 1982;42(11):4734-9.

Ganapathi R, Grabowski D, Sweatman TW, Seshadri R, Israel M. N-benzyladriamycin-14-valerate versus progressively doxorubicin-resistant murine tumours: cellular pharmacology and characterisation of cross-resistance in vitro and in vivo. Br J Cancer. 1989;60(6):819-26.

Gianni L, Herman EH, Lipshultz SE, Minotti G, Sarvazyan N, Sawyer DB. Anthracycline cardiotoxicity: from bench to bedside. J Clin Oncol. 2008;26(22):3777-84.

Herman EH, Rahman A, Ferrans VJ, Vick JA, Schein PS. Prevention of chronic doxorubicin cardiotoxicity in beagles by liposomal encapsulation. Cancer Res. 1983;43(11):5427-32.

Jones GL, Will A, Jackson GH, Webb NJ, Rule S; British Committee for Standards in Haematology. Guidelines for the management of tumour lysis syndrome in adults and children with haematological malignancies on behalf of the British Committee for Standards in Haematology. Brit J Haematol. 2015;169(5):661-71.

Kantarjian H. Acute myeloid leukemia—major progress over four decades and glimpses into the future. Am J Hematol. 2016;91(1):131-45.

Kolonias D, Podona T, Savaraj N, Gate L, Cossum P, Lampidis TJ. Comparison of annamycin to adriamycin in cardiac and MDR tumor cell systems. Anticancer Res. 1999;19(2A):1277-83.

Lilleby K, Garcia P, Gooley T, McDonnnell P, Taber R, Holmberg L, Maloney DG, et al. A prospective, randomized study of cryotherapy during administration of high-dose melphalan to decrease the severity and duration of oral mucositis in patients with multiple myeloma undergoing autologous peripheral blood stem cell transplantation. Bone Marrow Transplant. 2006:37(11):1031-5.

Mayhew E, Rustum Y, Vail WJ. Inhibition of liver metastases of M 5076 tumor by liposome-entrapped adriamycin. Cancer Drug Deliv. 1983;1(1):43-58.

Moscow JA, Cowan KH. Multidrug resistance. J Natl Cancer Inst. 1988;80(1):14-20.

Patel JP, Gonen M, Figueroa ME, Fernandez H, Sun Z, Racevskis J, et al. Prognostic relevance of integrated genetic profiling in acute myeloid leukemia. N Engl J Med. 2012;366(12):1079-89.

Perez-Soler R, Ling YH, Zou Y, Priebe W. Cellular pharmacology of the partially non-cross-resistant anthracycline annamycin entrapped in liposomes in KB and KB-V1 cells. Cancer Chemother Pharmacol. 1994;34(2):109-18.

Perez-Soler R, Neamati N, Zou Y, Schneider E, Doyle LA, Andreeff M, et al. Annamycin circumvents resistance mediated by the multidrug resistance-associated protein (MRP) in breast MCF-7 and small-cell lung UMCC-1 cancer cell lines selected for resistance to etoposide. Int J Cancer. 1997;71(1):35-41.

Perez-Soler R, Priebe W. Anthracycline antibiotics with high liposome entrapment: structural features and biological activity. Cancer Res. 1990;50(14):4260-6.

Priebe W, Van NT, Burke TG, Perez-Soler R. Removal of the basic center from doxorubicin partially overcomes multidrug resistance and decreases cardiotoxicity. Anticancer Drugs. 1993;4(1):37-48.

Rahman A, Carmichael D, Harris M, Roh JK. Comparative pharmacokinetics of free doxorubicin and doxorubicin entrapped in cardiolipin liposomes. Cancer Res. 1986;46(5):2295-9.

Wetzler M, Thomas DA, Wang ES, Shepard R, Ford LA, Heffner TL, et al. Phase I/II trial of nanomolecular liposomal annamycin in adult patients with relapsed/refractory acute lymphoblastic leukemia. Clin Lymphoma Myeloma Leuk. 2013;13:430-4.

Zamorano JL, Lancellotti P, Rodriguez Muñoz D, Aboyans V, Asteggiano R, Galderisi M, et al; ESC Scientific Document Group. 2016 ESC Position Paper on cancer treatments and cardiovascular toxicity developed under the auspices of the ESC Committee for Practice Guidelines. The Task Force for cancer treatments and cardiovascular toxicity of the European Society of Cardiology (ESC). Eur Heart J. 2016;37(36):2768-2801. Erratum in: Eur Heart J. 2018;39(10):839.

Zou Y, Ling YH, Van NT, PriebeW, Perez-Soler R. Antitumor activity of free and liposome-entrapped annamycin, a lipophilic anthracycline antibiotic with non-cross-resistance properties. Cancer Res. 1994;54(6):1479-84.

Zou Y, Priebe W, Stephens LC, Perez-Soler R. Preclinical toxicity of liposome-incorporated annamycin: selective bone marrow toxicity with lack of cardiotoxicity. Clin Cancer Res. 1995;1(11):1369-74.

APPENDIX A: SCHEDULE OF EVENTS

Event	Screening	Baseline[a]	On Treatment (Induction)								End of Study		Follow-up	Survival Follow-up
			First 3 Dosing Days			Weekly								
	≥Day -35	Day 1 (Prior to Dosing)	Day 1	Day 2	Day 3	Day 7	Day 14	Day 15	Day 18	Day 21 (At the End of the Induction Cycle)	Day 28	Day 35	Day 42	Q3 months
Informed consent	X													
Relapsed/refractory AML diagnosis	X													
ECOG performance status	X	X									X			
Medical history	X	X												
Physical examination[b]	X	X		X	X	X	X			X			X	
Electrocardiogram	X	X[i]		X[k]	X[k]	X[k]	X			X				
Blood chemistry[c]	X	X[i]		X	X	X	X			X	X			
CBC, differential, platelet count[c,d]	X	X[i]		X	X	X	X			X	X			
Urinalysis	X	X[i]												
Pregnancy test	X[e]					X[e]				X[e]				
MUGA, ECHO, or ECHO strain	X[f]								X[g]——— ———X					X[q]
Bone marrow aspirate and peripheral blood[h] for measuring disease		X[j]						X[j]——————————————————X						

Blood samples for pharmacokinetic analysis		X[k]	X[k]	X[l]	X[k,l]					X				
Blood samples for cardiac biomarkers		X[m]			X[m]					X				
Study drug administration			X[n]	X[n]	X[n]									
Prophylactic mouthwash			X[o]	X[o]	X[o]									
Antihistamine administration[p]			X	X	X									
Adverse event monitoring			X	X	X	X	X			X	X		X	X
Concomitant medication	X	X		X	X	X	X			X	X		X	

Abbreviations: AML = acute myeloid leukemia; BUN = blood urea nitrogen; CBC = complete blood count; CYP = Cytochrome P450 family of enzymes; DLT = dose-limiting toxicity; ECOG = Eastern Cooperative Oncology Group; ECG = electrocardiogram; ECHO = echocardiogram; LDH = lactate dehydrogenase; PK = pharmacokinetic; RP2D = recommended Phase 2 dose; SGOT = serum glutamic-oxaloacetic transaminase; SGPT = serum glutamic-pyruvic transaminase.

[a] Baseline (Day 1) assessments are to be completed prior to dosing on Day 1.
[b] Including height (screening only), weight, vital signs, and oral examination; this will occur daily for the first 3 days of drug administration.
[c] Sodium, potassium, chloride, bicarbonate, calcium, magnesium, phosphorus, BUN, creatinine, glucose, albumin, total protein, alkaline phosphatase, total bilirubin, SGOT, SGPT, LDH, and uric acid with creatinine clearance to be calculated from these results at screening; CBC with differential and platelet count; these evaluations will occur daily for the first 3 days of drug administration.
[d] CBC with differential and platelet count will be repeated if subject experiences a hematological toxicity that could be classified as a DLT after the first dose of the study drug.
[e] Pregnancy test for women of childbearing potential within 72 hours prior to first dose of study drug and on Day 7 and Day 21
[f] Within 2 weeks prior to the first dose of study drug
[g] A final MUGA or ECHO scan or an ECHO strain evaluation will be conducted between the Day 18 and Day 28 visits or at the the End of Treatment visit if the subject is prematurely terminated.
[h] For peripheral blood, include CBC, differential, and platelet count. In addition, flow cytometry analysis of peripheral blood may be utilized for measuring disease (optional). Bone marrow aspirate is sufficient if there are spicules present; if not, bone marrow biopsy is required (designated aspirate/biopsy below)
[i] CBC, blood chemistry, urinalysis, and ECGs should be repeated if screening was ≥14 days prior to first dose of study drug.
[j] Bone marrow aspirate and peripheral blood (complete blood count, differential, and platelet count; flow cytometry is optional) within 15 days prior to the first dose of liposomal annamycin to document disease status. Bone marrow aspirate/biopsy 15 to 35 days after the start of therapy will be performed. If cytogenetics were initially abnormal, include cytogenetics as part of the remission documentation.
[k] Blood samples for PK analysis collected at predose and at 0.25, 0.5, 1, 2, 4, 8, and 24 hours if clinically feasible and within a reasonable timeframe of the indicated times after the start of liposomal annamycin infusion on Day 1 and Day 3 for 3 subjects at each dose and 6 subjects at the RP2D who complete the

3 days of dosing. ECGs will also be collected for all subjects involved in pharmacokinetic analyses at predose and at 4 and 24 hours after dosing on Days 1 to 3, except for the 6 subjects at the RP2D who will have ECGs on Days 1 and 3 only. Documentation of concomitant medications affecting CYP enzymes will be obtained to ensure that these are a consideration in the evaluation of the pharmacokinetics of this drug.

[l] PK/ECG collection timepoint of 24 hours after the start of the infusion on Days 1 and 3, per above, will occur on Day 2 (predose) and Day 4.

[m] Blood sample for cardiac biomarkers (troponin-I and troponin-T) will be collected at predose and at the end of the infusion on Day 3.

[n] Liposomal annamycin is administered as a daily 2-hour intravenous infusion for 3 consecutive days followed by 18 days off liposomal annamycin (i.e., 1 treatment cycle = 21 days) every 3 weeks. Subjects will be hospitalized for the full 3 days of liposomal annamycin administration.

[o] Mouthwash per institution's guidelines and practices

[p] Antiallergic premedication with diphenhydramine at a dose of 50 mg administered intravenously must be administered before each dose of liposomal annamycin.

[q] A MUGA scan, ECHO scan, or ECHO strain will be collected at the Q3-month survival timepoint if possible.

APPENDIX B: EASTERN COOPERATIVE ONCOLOGY GROUP PERFORMANCE STATUS SCALE

Grade	Details
0	Fully active, able to carry on all predisease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light of sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all self care 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 self care. Totally confined to bed or chair.
5	Dead

APPENDIX C: COMMONLY USED CHEMOTHERAPY REGIMENS AND THEIR ANTHRACYCLINE CONTENT

Regimen	Anthracycline	Dose per course (mg/m^2)
ABVD	Doxorubicin	50
AC	Doxorubicin	60
Baseline BEACOPP	Doxorubicin	25
CHOP (R_CHOP)	Doxorubicin	50
Cisplatin/Eirubicin	Epirubicin	90
CODOX-M	Doxorubicin	40
D(90)A 3+7	Daunorubicin	270
D(60)A 3+7	Daunorubicin	180
DA 3+	Daunorubicin	150
DA 2+5	Daunorubicin	100
DAE	Daunorubicin	150
Dclo	Daunorubicin	150
Esc BEACOPP	Doxorubicin	35
EC90	Epirubicin	90
Epi-CEM	Epirubicin	150
FEC75	Epirubicin	75
FEC100	Epirubicin	100
FLA(G)-Ida	Idarubicin (IV)	24
FMD	Mitoxantrone	12
Hyper-CVAD	Doxorubicin	50
Ida-Ara-c	Idarubicin (IV)	30
IDARAM	Idarubicin (IV)	20
IVE	Epirubicin	50
MAXI-CHOP	Doxorubicin	75
MCD	Mitoxantrone	12
MiDAC	Mitoxantrone	50
PAD	Doxorubicin	36
Stanford V	Doxorubicin	25
VAD	Doxorubicin	36
Z-DEX	Idarubicin (PO)	40
Vyxeos	Daunorubicin	132

Abbreviations: IV = intravenous; PO = per os (orally).

APPENDIX D: DEFINITION OF HY'S LAW

- The drug causes hepatocellular injury, generally defined as an elevated alanine aminotransferase or aspartate aminotransferase by 3-fold or greater above the upper limit of normal.
- Among subjects showing such aminotransferase elevations, they also have elevation of their serum total bilirubin of greater than 2 × the upper limit of normal, without findings of cholestasis (defined as serum alkaline phosphatase activity less than 2 × the upper limit of normal).
- No other reason can be found to explain the combination of increased aminotransferase and serum total bilirubin, such as viral hepatitis, alcohol abuse, ischemia, preexisting liver disease, or another drug capable of causing the observed injury.

Reference: Guidance for Industry Drug-Induced Liver Injury: Premarketing Clinical Evaluation [Internet]. Rockville: U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research (CDER) Center for Biologics Evaluation and Research (CBER); c2009 [cited 2019 Feb 17] Available from: https://www.fda.gov/downloads/Drugs/.../Guidances/UCM174090.pdf

APPENDIX E: CALCULATIONS OF ANTHRACYCLINE EXPOSURE

Cardioprotective Measures for Anthracycline Drugs (adapted from Zamorano et al. 2016)

Potential Cardioprotective Measures for Anthracycline Drugs and Analogues by Limitation of Cumulative Doses	
Drug	**Conversion Factor (Daunorubicin Equivalents)**
Daunorubicin	1
Doxorubicin	2.2
Epirubicin	1.1
Mitoxantrone	5.0
Idarubicin	5.3
Liposomal Annamycin	1

All daunorubicin equivalents are based on <551 mg/m^2 as being the maximal allowable dose with the maximal exposure of anthracyclines capped at 550.9 mg/m^2. This will be calculated as a daunorubicin equivalent added to the anticipated exposure to liposomal annamycin. The calculations and results will be entered into the appropriate eCRF page.

Additional References:

Alderton PM, Gross J, Green MD. Comparative study of doxorubicin, mitoxantrone, and epirubicin in combination with ICRF-187 (ADR-529) in a chronic cardiotoxicity animal model. Cancer Res. 1992;52(1):194-201.

Anderlini P, Benjamin RS, Wong FC, Kantarjian HM, Andreeff M, Kornblau SM, et al. Idarubicin cardiotoxicity: a retrospective study in acute myeloid leukemia and myelodysplasia. J Clin Oncol. 1995;13(11):2827-34.

Avilés A, Neri N, Nambo JM, Huerta-Guzman J, Talavera A, Cleto S. Late cardiac toxicity secondary to treatment in Hodgkin's disease. A study comparing doxorubicin, epirubicin and mitoxantrone in combined therapy. Leuk Lymphoma. 2005;46(7):1023-8.

Feijen EA Leisenring WM, Stratton KL, Ness KK, van der Pal HJ, Caron HN, et al. Equivalence ratio for daunorubicin to doxorubicin in relation to late heart failure in survivors of childhood cancer. J Clin Oncol. 2015;33(32):3774-80.

Lefrak EA, Pitha J, Rosenheim S, Gottlieb JA. A clinicopathologic analysis of adriamycin cardiotoxicity. Cancer. 1973;32(2):302-14.

McGowan JV, Chung R, Maulik A, Piotrowska I, Malcolm Walker J, Yellon DM. Anthracycline chemotherapy and cardiotoxicity. Cardiovasc Drugs Ther. 2017;31:63-75.

Ryberg M, Nielsen D, Skovsgaard T, Hansen J, Jensen BV, Dombernowsky P. Epirubicin cardiotoxicity: an analysis of 469 patients with metastatic breast cancer. J Clin Oncol. 1998;16(11): 3502-8.

Takemura G, Fujiwara H. Doxorubicin-induced cardiomyopathy from the cardiotoxic mechanisms to management. Prog Cardiovasc Dis. 2007;49:330-52.

Von Hoff DD, Rozencweig M, Layard M, Slavik M, Muggia FM. Daunomycin-induced cardiotoxicity in children and adults a review of 110 cases. Amer J Med. 1977;62:200-8.