A Phase Ib/II Clinical Trial of Oral Ciprofloxacin and Etoposide in Subjects with Resistant Acute Myeloid Leukemia (AML)

Protocol Number:	UF-HEM-005
Principal Investigator:	Randall A. Brown, M.D. University of Florida 1600 SW Archer Road Box 100278 Gainesville, FL 32610-0278 Email: randy.brown@medicine.ufl.edu Office: 352-273-7832
Statistical Support:	Ji-Hyun Lee Director, Division of Quantitative Sciences University of Florida Health Cancer Center Professor, Department of Biostatistics University of Florida Gainesville, FL Email: jihyun.lee@ufl.edu Phone: 352-273-9079
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ABBREVIATIONS

AE	adverse event
ALT	alanine transaminase (also SGPT)
AML	acute myeloid leukemia
ANC	absolute neutrophil count
APL	acute promyelocytic leukemia
AST	aspartate transaminase (also SGOT)
AUC	area under curve
$AUC_{(0-t)}$	area under the plasma concentration-time curve from time zero to time t
$AUC_{(0-\infty)}$	area under the plasma concentration-time curve from time zero to infinity
$AUC_{(0-Tlast)}$	area under the plasma concentration-time curve from time zero to the last measurable time point
BAD	biologically active dose
BSA	body surface area
BUN	blood urea nitrogen
C	cycle
CBC	complete blood count
CL	clearance
CLB_{oralB}	apparent clearance after oral administration
CB_{maxB}	maximum plasma concentration
CMP	comprehensive metabolic panel
CR	complete remission
CRi	complete remission (with incomplete blood counts)
CRp	complete remission (with incomplete platelet recovery)
CRF	case report form
CTCAE	Common Terminology Criteria for Adverse Events
D	Day
DISC	Data Integrity and Safety Committee
dL	deciliter
DLT	dose-limiting toxicity
DNA	deoxyribonucleic acid
ECG	electrocardiogram
ECOG	Eastern Cooperative Oncology Group
EMD	extramedullary disease
FISH	fluorescence in situ hybridization
GCP	Good Clinical Practice
GI	gastrointestinal
HI	hematologic improvement (see Appendix D)
HMA	Hypomethylating agent
ICF	informed consent form

ICH	International Conference on Harmonization
IPSS	International Prognostic Scoring System
IRB	Institutional Review Board
IV	intravenous
IWG	International Working Group
Ke	elimination rate constant
LC-MS/MS	high performance liquid chromatography/tandem mass spectrometry
LDH	lactic dehydrogenase
MCH	mean corpuscular hemoglobin
MCHC	mean corpuscular hemoglobin concentration
MCV	mean corpuscular volume
MDS	myelodysplastic syndromes
mg	milligram
MTD	maximum tolerated dose
NOAEL	no observed adverse effect level
OS	overall survival
PCR	polymerase chain reaction
PFS	progression free survival
PI	principal investigator
PML	promyelocytic leukemia
PR	partial remission
PT	prothrombin time
PTT	partial thromboplastin time
RBC	red blood cells
RNA	ribonucleic acid
SAE	serious adverse event
SC	subcutaneous
SD	stable disease
SGOT	serum glutamic oxaloacetic transaminase (also AST)
SGPT	serum glutamic pyruvate transaminase (also ALT)
t½	terminal elimination half-life
T_{max}	time to maximum plasma concentration
UF	University of Florida
UFHCC	University of Florida Health Cancer Center
ULN	upper limit of normal
US	United States
WBC	white blood cell
WHO	World Health Organization

PROTOCOL SYNOPSIS

Title:	**A Phase Ib/II Clinical Trial of Oral Ciprofloxacin and Etoposide in Subjects with Resistant Acute Myeloid Leukemia (AML)**
Rationale:	There is a paucity of treatment options for subjects with resistant AML. Recently AML has been shown to be a multi-genetic disease with sub-clonal architecture that evolves spontaneously and in response to treatment. Chemotherapy-resistant leukemic clones can quickly gain dominance by "survival of the fittest" dynamics. One way of targeting minor yet resistant clones is to augment the effectiveness of current chemotherapy drugs. Hromas et al. discovered the importance of the Metnase complex in repairing chemotherapy related DNA damage in leukemia and other cancers (1-3). Screening studies identified ciprofloxacin as a Metnase inhibitor that chemosensitizes leukemic myeloblasts to etoposide chemotherapy (4). The non-overlapping toxicity profiles of ciprofloxacin and etoposide, and their additive effectiveness in this model, make these agents attractive candidates for use in combination in subjects with resistant AML.
Primary Objective:	**In subjects with resistant AML, to** • Phase 1b: Establish the maximum tolerated dose (MTD) of oral ciprofloxacin when given in combination with a fixed dose of oral etoposide to subjects with resistant AML. • Phase II: Determine the complete response (CR, CRi, and CRp, see Appendix A) rate to treatment with ciprofloxacin and etoposide at the MTD in adults with resistant AML.
Secondary Objectives:	**In subjects with resistant AML, to** • Determine the safety and feasibility of treatment with oral ciprofloxacin and etoposide; • Measure duration of complete response, of progression free survival and of overall survival following treatment with oral ciprofloxacin and etoposide; and • Estimate the incidence of Grade ≥ 3 adverse events following treatment with ciprofloxacin and etoposide.
Study Design:	This is an open-label, single arm, **Phase Ib/II** study of oral ciprofloxacin and etoposide in subjects with resistant AML. This study is designed to evaluate the complete response rate, the safety profile, and biologic activity of the combination of oral ciprofloxacin and etoposide in subjects with resistant AML. For both study phases, all subjects will receive 1 cycle of induction. At the end of Cycle 1, subjects who demonstrate progressive disease will have completed study treatment. For any subject who demonstrates stable disease or a

partial response, a second cycle of induction will be given. No subject will receive more than 2 cycles of induction.

Phase Ib:

In the **Phase Ib** portion of the study, subjects will be enrolled in cohorts of three (3) in a traditional 3+3 study design (5). The starting dose is level "0" and includes: ciprofloxacin 750 mg orally twice daily on Days 1 - 10 and etoposide 200 mg orally once daily on Days 2 – 8. During the first cycle of treatment, subjects will be individually assessed for safety and dose limiting toxicity (DLT) for the purpose of determining the MTD of ciprofloxacin. The MTD will be defined as the highest dose of ciprofloxacin at which < 33% of the subjects treated at a given dose level experience a DLT (See Figure 1**)**. The **Phase Ib** portion of this study ends when a MTD is identified among the three planned dose levels (See Table 2).

Subjects withdrawn before Cycle 1 Day 1 (C1D1) will not be evaluable for dose escalation decisions and will be replaced in their treatment cohort. A subject is evaluable for dose-escalation decisions provided: they receive all drug doses and remain on study until evaluation at Cycle 1 Day 28 without a DLT; or they experience a DLT during Cycle 1. Subjects who miss any etoposide or ciprofloxacin doses or withdraw from study without a DLT and prior to Cycle 1 Day 28 are not evaluable for dose escalation decisions and will be replaced in their cohort of treatment.

During dose escalation no more than 3 subjects will be treated simultaneously at any given dose level. The **Phase Ib** portion of this study will not be complete until > 28 days after accrual of the last subject.

Phase II:

Enrollment in the **Phase II** portion of the study will begin once the MTD has been determined. After Cycle 1, subjects with progressive disease will be removed from the study. Other study participants will continue to receive a second 28-day cycle of ciprofloxacin and etoposide at the MTD or until one of the following occurs: disease progression, unacceptable toxicity, consent withdrawal, completion of Cycle 2, or termination of the study.

Dose Limiting Toxicity (DLT)

DLT will be defined as any of the following events that are considered to be related to treatment with ciprofloxacin and etoposide. For the purpose of dose-escalation and MTD determination, only DLTs that occur during the first cycle of oral ciprofloxacin and etoposide will be considered. These include:

1. Any Grade ≥ 3 non-hematologic toxicity, except alopecia; and vomiting or nausea uncontrolled by medical management;

	2. Any treatment-related effect that results in a subject missing more than 25% of ciprofloxacin or etoposide doses; 3. Any non-hematologic toxicity considered to be related to ciprofloxacin or etoposide that results in a delay of Cycle 2 by > 14 days; 4. Grade 4 neutropenia (absolute neutrophil count < 500/mm^3) in subjects with baseline ANC levels > 500/mm^3 persisting up to Day 42 after the start treatment, in the absence of leukemia; 5. Grade 3 or greater QTc prolongation developing after the start of therapy with oral ciprofloxacin; and/or 6. Any episode of tendon rupture. Prior to commencement of Phase II, DLT's and records of toxicity will be reviewed by the Data Integrity and Safety Committee (DISC).
Inclusion/ Exclusion Criteria:	**Inclusion criteria - All of the following must apply:** A. Age ≥ 18 years. B. Diagnosis of AML confirmed by review of bone marrow pathology at the University of Florida. C. Patients with relapsed and or refractory AML • who failed to achieve CR or CRi after at least one cycle of induction chemotherapy and not suitable for second cycle of standard intensive chemotherapy, • who have progressed after 1 cycle of HMA or intolerant to HMA therapy and not suitable for standard induction chemotherapy regimens. • who have relapsed after any duration of response, D. Per the treating physician, the subject must have a life expectancy of ≥ 4 weeks. E. Subject performance status must be ECOG 0, 1, or 2 (See Appendix B). F. Subject must have a total bilirubin ≤ 2 mg/dL and AST and ALT ≤ 2.5 times upper limit of normal. G. Subject must have serum creatinine < 2 mg/dL. H. Females of child-bearing potential (*i.e.*, women who are pre-menopausal or not surgically sterile) may participate, provided they meet the following conditions: o Must agree to use physician-approved contraceptive methods throughout the study and for 6 months following the last dose of ciprofloxacin and/or etoposide. Adequate forms of contraception are abstinence, double barrier methods (*e.g.,* condoms with spermicidal jelly or foam and diaphragm with spermicidal jelly or foam), oral, depot, or injectable contraceptives, intrauterine devices, and tubal ligation. o Must have a negative serum pregnancy test within 7 days prior to beginning treatment on this study.

	I. Males with female partners of child-bearing potential must agree to use physician-approved contraceptive methods (*e.g.,* abstinence, condoms, vasectomy) throughout the study and should avoid conceiving children for 6 months following the last dose of ciprofloxacin and/or etoposide. J. Subjects must have provided written informed consent, and demonstrated the willingness and ability to comply with all study-related procedures. **Exclusion criteria - None of the following can apply:** A. History of allergic or significant adverse reaction (*e.g.*, anaphylaxis, prolonged QTc, or severe tendonitis) to ciprofloxacin or etoposide. B. Acute promyelocytic leukemia (APL) with t(15;17). C. Prolonged baseline QTc, defined as QTc interval > 470 msec in women and > 450 msec in men, or > 480 msec for subjects with a bundle branch block. D. Uncontrolled clinically significant infection. Subjects with fever (temperature $\geq 38.3^0$C) thought to be related to leukemia are eligible assuming that blood cultures are negative during the 7 days prior to Cycle 1 Day 1 and there is no clinical evidence of active infection (*e.g.,* negative or stable radiographs and negative physical examination). E. Ongoing, symptomatic *Clostridium difficile* infection. Subjects who are asymptomatic with negative stool for C. difficile may participate. F. Pregnant and/or nursing. G. History of Myasthenia Gravis. H. Treatment with any anticancer therapy (standard or investigational) within 14 days prior to the first dose of ciprofloxacin or less than full recovery ($\leq$ CTCAE Grade 1) from the clinically significant toxic effects of that treatment. The use of hydroxyurea is allowed only during the first 14 days of cycle 1.
Study Duration and Dates:	• It is estimated that the subject recruitment period will be approximately 48 months. No subject will receive more than 2 cycles of therapy from Day 1 of Cycle 1. • Data regarding disease status and overall survival will be collected monthly until disease progression, death or until 3 years from the on-study date.

Safety Assessments:	Subjects will be evaluated weekly during and for at least 28 days following the last cycle of chemotherapy. Assessments will include (See Table 1): • Interval history, review of systems, medications, new allergies, and physical examination with vital sign measurements; • Weekly laboratory assessments, including a CBC with differential and CMP; and • Toxicity assessment per the NIH Common Toxicity Criteria Adverse Event (CTCAE) version 4.
Efficacy Assessments:	Efficacy parameters used to assess response include hemoglobin, ANC, platelet count, circulating blasts, and bone marrow aspirate and/or biopsy.
Sample Size Estimation	In prior clinical trials of oral etoposide in resistant AML the CR rate has been approximately 20%. It is expect that the combination of etoposide with ciprofloxacin will improve CR to 40%. In order to detect this difference with type I error rate of 0.10 and power of 80%, when the true response rate is 40%, a minimum of 24 evaluable subjects will be treated in Phase II based on the Simon's two-stage MiniMax design. Accounting for 15-20% drop out rate, a total of 30 subjects will need to be recruited to this phase.

Table 1. Study Events Schedule

Assessment	Screen (≤ 21 days prior to Day 1)	Cycle 1					Cycle 2		End of Treat-ment[12] ± 7days	Long-Term Follow Up[13]
		Day 1	Day 8±2	Day 15±2	Day 22±2	Day 28±2	Days 1, 8, 15, 22±2	Day 28±2		
Signed Informed Consent	X									
Demographics	X									
Medical /Treatment History	X									
Medication History[1]	X	X	X	X	X	X	X	X	X	
Interval History/Physical Examination	X	X	X	X	X	X	X	X	X	
Toxicity Assessment		X	X	X	X	X	X	X	X	
Review of Systems		X	X	X	X	X	X	X	X	
Weight	X	X	X	X	X	X	X	X	X	
ECOG Performance Status	X	X	X	X	X	X	X	X	X	
12-lead electrocardiogram (ECG)[2]	X		X			X			X	
Vital Signs[3]	X	X	X	X	X	X	X	X	X	
Hematology Laboratory Evaluations[4]	X	X	X	X	X	X	X	X	X	
Blood Chemistry Laboratory Evaluations[5]	X	X	X	X	X	X	X	X	X	
Serum Pregnancy Test (females)[6]	X					X		X	X	
Administer Ciprofloxacin[7]		X	X				X			
Administer Etoposide[8]			X				X			
Bone Marrow Aspirate and/or Biopsy[9]	X					X		X	X	
Adverse Events Assessments[11]	X	X	X	X	X	X[13]	X	X	X	
Survival Assessment										X

[1] Document all medications taken during the two (2) weeks prior to screening and throughout and for 28 days after last treatment.
[2] Includes: rhythm, rate, and PR, QRS, and QTc intervals.
[3] Includes: systolic and diastolic blood pressure, pulse rate, respiratory rate, and temperature.
[4] Complete CBC including: hemoglobin, hematocrit, platelet and white blood cell count (WBC) with differential including circulating blasts and absolute neutrophil count.
[5] BUN, creatinine, uric acid, total bilirubin, AST, ALT, alkaline phosphatase, LDH, sodium, potassium, calcium, magnesium, phosphorous, bicarbonate, chloride, glucose, albumin, and total protein.
[6] Serum pregnancy test within 1 week of Cycle 1 Day 1 for females of childbearing potential. Repeat within one week prior to first day of each subsequent cycle.
[7] Ciprofloxacin will be administered orally twice daily on Days 1 to 10 of a 28-day cycle.
[8] Etoposide 200 mg will be administered orally once daily on Days 2 to 8 of a 28-day cycle.
[9] The screening bone marrow assessment must be performed within 42 days of Cycle 1 Day 1 and must be reviewed at UF. If bone marrow analysis results from outside lab is used to confirm diagnosis, slides must be made available to be read by UF pathologist. This must confirm a diagnosis of AML, non-M3. In case of archived marrow used for eligibility or bone marrow inaspirable or slides unavailable to be read by UF pathologist, and there is greater than 10% blast present, peripheral blood may be collected for flow cytometry.
[11] Monitoring for SAEs and AEs starts the day patient signs the informed consent form (ICF), continuing throughout the study. Subjects who have received at least one dose of study drug and discontinue prematurely (regardless of reason) will be followed for any adverse events that occur during the study until 28 days (± 2 days) following the last dose of study treatment (i.e., the Follow-up Visit).
[12] End of treatment visit will occur at C1D28 visit in subjects with progressive disease or at study withdrawal if the date does not correspond to a protocol-specified visit. Additional follow-up will include an assessment for the evaluation of AEs and concomitant medications 28 days (± 7 days) after the last dose of study medication. Other assessments will be performed as necessary by the treating physician.
[13] All subjects will be followed monthly until: disease progression, death, or until 3 years from the on-study date.

1. BACKGROUND AND RATIONALE

1.1 Management of Resistant Acute Myeloid Leukemia (AML)

Patients with AML who fail induction chemotherapy (refractory) and those who relapse after optimal chemotherapy are seldom cured with salvage regimens (6). While allogeneic stem cell transplantation can cure 30-50% of patients with resistant AML who are transplanted in complete remission (CR), most patients are not candidates due to failure to achieve CR with salvage therapy or due to comorbid conditions or absence of a suitable donor.

Given that resistance to chemotherapy is the major cause of death for patients with AML, there is an urgent need for novel treatment regimens that are well tolerated and capable of overcoming disease resistance (7). Recent advances in molecular genetics have revealed that AML is characterized by a complex, sub-clonal architecture that evolves spontaneously and in response to treatment. Chemotherapy-resistant leukemia clones can quickly become dominant by "survival of the fittest" dynamics. In order to target minor, yet resistant clones, one strategy is to augment the effectiveness of current chemotherapeutic agents.

1.2 Etoposide (VP-16) in AML

Etoposide is a semisynthetic glucoside derivative of podophyllotoxin. Although the exact mechanism of action is uncertain, it is generally accepted that etoposide acts as a Topo II inhibitor triggering decatenation checkpoint arrest (2). Although intravenous etoposide has activity in a variety of hematologic malignancies and solid tumors, the drug has usually been combined with other active agents so that the contribution of etoposide to efficacy or toxicity is difficult to assess (8-11). However, high-dose single-agent etoposide is clearly active in patients with hematologic malignancies although toxicity is severe (12).

Oral etoposide was developed to facilitate outpatient therapy. In addition, available data suggests schedule dependency for etoposide such that administration on multiple consecutive days may be more effective than dosing at 6 to 8 day intervals (13). Availability of oral etoposide permits prolonged exposure without multiple office visits. Perhaps the largest experience using single agent oral etoposide in patients with hematologic malignancies was reported by Osby et al (14) . In that trial etoposide was given at a fixed dose of 100 mg daily for 14 days in 21 day cycles. As expected, myelosuppression was the major toxicity with approximately 2/3 of all patients experiencing grade 3/4 neutropenia. Of 13 patients with AML (11) or myelodysplasia (2), 5 received a single course due to severe, primarily hematologic toxicity. Non-hematologic toxicity included grade 2 hepatic dysfunction in 2/14 evaluable patients, grade 1 renal dysfunction in 1/11 patients and grade 3 nausea/vomiting in 2/14 patients. This toxicity profile is similar to that described by others following treatment with multiple daily doses of oral etoposide with the exception of mucositis described with 21 day schedules (13) .

In conclusion, oral etoposide is an active and well tolerated agent for treatment of resistant AML and offers the convenience of treatment in the outpatient setting.

1.3 Metnase: A DNA Repair Component Mediates Resistance to Topo II Inhibitors

DNA double strand breaks can be generated by failed decatenation and Topoisomerase IIα (Topo II) is the critical decatenating enzyme (2). Topo II inhibitors, such as etoposide, can trigger decatenation checkpoint arrest at either of 2 decatenation mitotic checkpoints. Metnase (also

SETMAR) is a novel DNA repair protein with a SET histone methylase domain and a transposase nuclease domain and both are essential for enhancement of DNA double-strand break repair. Hromas et al reported that both AML and ALL cells fail to arrest in mitosis when Topo II is inhibited and that Metnase levels mediate progression through mitosis by enhancing Topo II function. Critically, Metanse mediates cell proliferation through and protects Topo II from etoposide. This implies that Metanse is a critical mediator of leukemia resistance to the cytotoxic effect of Topo II inhibitors (1).

Based on these observations, inhibition of Metnase could overcome the resistance of leukemia cells to Topo II inhibitors. Small molecule inhibition of Metnase should show an excellent therapeutic index given that it is overexpressed in malignant cells and that there are few other human Transposase domain proteins with which to cross-react. Hromas et al virtually screened a large chemical library of small compounds for docking in the into the Metnase nuclease active site (4). It was found that high but clinically achievable concentrations of ciprofloxacin blocked the ability of Metnase to cleave DNA, which is essential for its ability to repair DNA double-strand breaks. Further, ciprofloxacin enhanced the antiproliferative effects of etoposide on the AML cell line KG-1(4).

In summary, ciprofloxacin is a Metnase inhibitor which enhances the antiproliferative effect of etoposide on AML cells in-vitro. These observations indicate that ciprofloxacin could improve the effectiveness of etoposide in the treatment of resistant AML.

1.4 Proposal: Treatment of Resistant AML with Etoposide and Ciprofloxacin

This protocol proposes to treat patients with resistant AML using the combination of etoposide and ciprofloxacin. In the Phase Ib portion of this trial, the maximum tolerated dose will be determined of ciprofloxacin when given in combination with a fixed dose of etoposide. The protocol will then proceed with the Phase II portion, in which the activity of this combination will be determined in patients with resistant AML.

2. OBJECTIVES

2.1 Primary

In subjects with resistant AML, to

Phase Ib: Establish the maximum tolerated dose (MTD) of oral ciprofloxacin when given in combination with a fixed dose of oral etoposide.

Phase II: Determine the rate of CR (See Appendix A) following treatment with the MTD (15).

2.2 Secondary

In subjects with resistant AML, to

- Determine the safety and feasibility of treatment with ciprofloxacin and etoposide;
- Measure the response duration, progression-free and overall survival (PFS and OS respectively);
- Estimate the incidence of Grade ≥ 3 adverse events following treatment with ciprofloxacin and etoposide at the MTD.

3. STUDY DESIGN

3.1 Overview

This is an open label, single arm, phase Ib/II study of oral ciprofloxacin and etoposide in subjects with resistant AML. In the Phase Ib portion of the study, the MTD of ciprofloxacin will be determined using a standard 3+3 design. In the Phase II portion the rate of CR will be estimated following treatment with a fixed dose of etoposide given with the MTD of ciprofloxacin. For both study phases, all subjects will receive 1 cycle of induction. At the end of Cycle 1, subjects who demonstrate progressive disease will have completed study treatment. For any subject who demonstrates stable disease or a partial response, a second cycle of induction will be given. No subject will receive more than 2 cycles of induction.

3.2 Rationale for Drug Doses and Schedule

3.2.1 Etoposide

As discussed in Section 1.2, oral etoposide is an active agent in the treatment of AML. Etoposide absorption is saturable at doses > 200 mg/day. Lower doses are associated with increased bioavailability but with higher inter- and intra-subject variability (16). In the current study etoposide will be administered at a daily dose of 200 mg in order to optimize bioavailability. As discussed above, the use of 14 day cycles of oral etoposide resulted in toxicity which limited therapy to a single cycle in over 1/3 of those treated (14). In order to give multiple cycles of therapy 7-day courses of etoposide will be administered.

3.2.2 Ciprofloxacin

Ciprofloxacin is a quinolone antibiotic which works by inhibiting DNA gyrase (17, 18). Ciprofloxacin is a Topo II inhibitor and in-vitro and xenograft tumor models have shown that this drug has anti-proliferative and possibly pro-apoptotic activity (19-23) at concentrations ranging from 12-800 mcg/ML (19, 20, 23-27) . The use of ciprofloxacin in the current trial is based on the ability of this drug to inhibit Metnase and thereby enhance the activity of etoposide in subjects with resistant acute leukemia. Hromas et al demonstrated that ciprofloxacin potentiated the activity of etoposide on AML cell lines at a concentration of 2 µmol/L (0.662µg/mL based on its molecular weight of 331) (4).

Per the prescribing information, a single dose of 250 mg, 500 mg, 750 mg, and 1000 mg of oral ciprofloxacin in healthy volunteers achieved a Cmax of 1.2, 2.4, 4.3 and 5.4 µg/mL (28). The elimination half-life of ciprofloxacin is 4 hours, and PK studies have shown the mean serum concentrations at 12 hours after a single dose of 250 mg, 500 mg, and 750 mg were 0.1, 0.2 and 0.4 µg/mL, respectively.

The 750 mg twice daily as a starting dose was chosen because this dose results in sustained levels that approximate those shown to augment etoposide effect (See Table 2) (4) . Further, this dose is commonly used for treatment of infection and has been well tolerated (28). To achieve a steady state concentration prior to initiation of etoposide, ciprofloxacin will be started 24 hours before etoposide is begun. If the starting dose of ciprofloxacin is tolerated, a higher dose of 1,000 mg twice daily will be explored in order to further increase ciprofloxacin 12 hour levels. Although less commonly used, this dose has also been well tolerated in studies in normal volunteers and in subjects with infection (29, 30).

3.3 Phase Ib

3.3.1 Dose Escalation and De-Escalation

The dose escalation phase of the study will utilize a standard "3+3" design to establish the MTD of oral ciprofloxacin in combination with a fixed dose of etoposide (200 mg daily on days 2-8). Ciprofloxacin will be given on days 1-10 at one of three dose levels shown in Table 2. The MTD will be defined as the level associated with a < 1/3 chance of DLT. To summarize: (See Figure 1)

1) Three subjects are initially treated at dose Level 0 (ciprofloxacin 750 mg twice daily).
   a) If none of these experience DLT, then dose will be escalated to Level +1.
   b) If one of these experiences DLT, then up to three more subjects are treated at Dose Level 1. If one or more of these develops DLT, then the MTD is considered to be exceeded and dose will be reduced to Level -1. If none of these additional subjects develop a DLT then the dose will be escalated to Level +1.
   c) If 2/3 of these subjects experience DLT, then the MTD is considered to be exceeded and dose is reduced to Dose Level -1.
2) If the dose is escalated to Dose Level +1, the same process will be followed to determine if this is the MTD (no further escalation).
3) If the dose is reduced to Level -1, then the same process will be followed to determine if this is the MTD. If Dose Level -1 is above the MTD the study will be closed and it will be concluded that treatment with this combination at effective doses is not safe.

Table 2. Planned Dose Levels

Dose Level	Drug	Dose	Frequency	Schedule
0	Ciprofloxacin Etoposide	750mg 200mg	Twice Daily Once Daily	D1-10 D2-8
+1	Ciprofloxacin Etoposide	1000mg 200mg	Twice Daily Once Daily	D1-10 D2-8
-1	Ciprofloxacin Etoposide	500mg 200mg	Twice Daily Once Daily	D1-10 D2-8

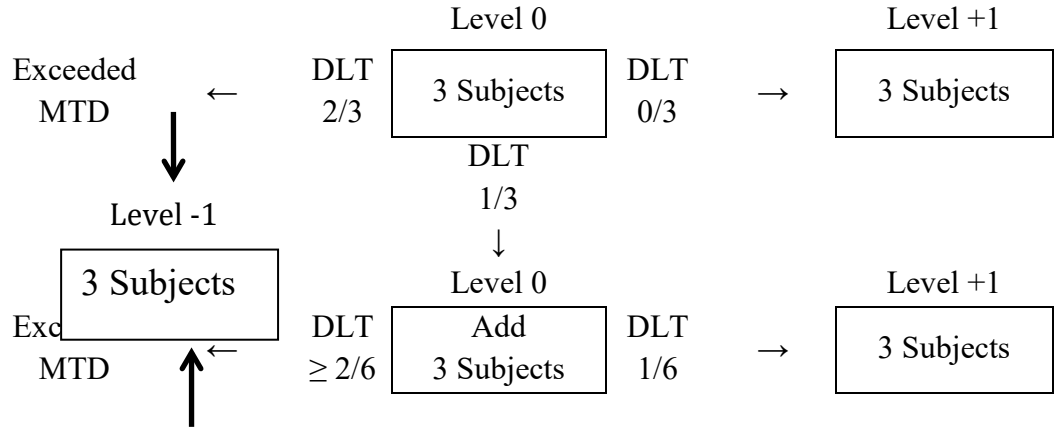
Subjects withdrawn before Cycle 1 Day 1(C1D1) will not be evaluable for dose escalation decisions and will be replaced in their treatment cohort. A subject is evaluable for dose-escalation decisions provided: they receive all drug doses and remain on study until evaluation at Cycle 1 Day 28 without a DLT; or they experience a DLT during Cycle 1. Subjects who miss any etoposide or ciprofloxacin doses or withdraw from study without a DLT and prior to Cycle 1, day 28 are not evaluable for dose escalation decisions and will be replaced in their cohort of treatment.

No more than 3 subjects will be added simultaneously to a dose cohort during dose escalation. The Phase Ib portion of this study ends when the MTD has been identified.

Subjects entered during Phase 1b who develop DLT with the first cycle will be withdrawn from the study. Subjects who do not develop DLT with the first cycle, will be treated in a second repeated 28-day cycle with ciprofloxacin and etoposide at the dose level given during Cycle 1

until completion of Cycle 2, disease progression, unacceptable toxicity, consent withdrawal, PI decision to discontinue treatment, or termination of the study. Intra-subject dose escalation will not be permitted. No subject will receive more than 2 cycles of treatment.

Figure 1. Example of How MTD Will Be Determined in Cycle 1



3.3.2 Dose Limiting Toxicity

DLT will be defined as any of the following events determined by the PI to be related to treatment with ciprofloxacin and etoposide and that constitute a change from baseline irrespective of outcome. For the purpose of dose-escalation decisions and MTD determination, only DLTs that occur during the first cycle of ciprofloxacin and etoposide treatment will be taken into account.

1. Any Grade > 3 non-hematologic toxicity, except alopecia; and vomiting or nausea uncontrolled by medical management;
2. Any treatment-related effect that results in a subject missing more than 25% of ciprofloxacin or etoposide;
3. Any non-hematologic toxicity considered to be related to ciprofloxacin or etoposide that results in a delay of Cycle 2 by > 14 days;
4. Grade 4 neutropenia (absolute neutrophil count < 500/mm^3) in subjects with baseline ANC levels > 500/mm^3 persisting up to 42 days after the start of treatment, in the absence of leukemia;
5. Grade 3 or greater QTc prolongation developing after the start of therapy with oral ciprofloxacin; and/or
6. Any episode of tendon rupture.

For the purpose of dose-escalation/de-escalation decisions and MTD determination, only DLTs that occur during Cycle 1 of Phase Ib will be considered. DLT assessment will occur at the Cycle 1 Day 28 visit. Prior to commencement of Phase II, DLT's and records of toxicity will be reviewed by the DISC.

3.4 Phase II

Enrollment in the Phase II portion of the study (dose expansion) will begin once the MTD has been determined. After Cycle 1, subjects with progressive disease will be removed from study. Others will continue to receive 28-day cycles of ciprofloxacin and etoposide at the same dose level until completion of Cycle 2, disease progression, unacceptable toxicity, consent

withdrawal, PI decision to discontinue treatment, or termination of the study. No subject will receive more than 2 cycles of therapy.

3.5 Study Duration

The subject recruitment period is approximately 48 months. The study duration encompasses the following:

- Screening period of up to 21 days;
- Treatment with ciprofloxacin and etoposide until the endpoints defined under Section 3.4 are met; and
- Day 28 follow-up after the last cycle of therapy.
- No subject will receive more than 2 cycles of therapy from Day 1 of Cycle 1.

3. SELECTION OF SUBJECTS

3.1 Number of Subjects

The number of subjects enrolled in Phase Ib to be 8-24 depending upon toxicity, after accounting for a 25% drop-out rate during the first 28-day cycle. In Phase II, up to 44 subjects with relapsed and/or refractory AML will be enrolled. Justification for sample size in phase II is provided in Section 11.1. Subjects in this study can be of any race, gender and ethnic group.

3.2 Inclusion Criteria

A. Age ≥ 18 years.

B. Diagnosis of AML confirmed by review of bone marrow pathology at the University of Florida.

C. Patients with relapsed and or refractory AML

- who failed to achieve CR or CRi after at least one cycle of induction chemotherapy and not suitable for second cycle of standard intensive chemotherapy,
- who have progressed after 1 cycle of HMA or intolerant to HMA therapy and not suitable for standard induction chemotherapy regimens.
- who have relapsed after any duration of response,

D. Per the treating physician, the subject must have a life expectancy of ≥ 4 weeks.

E. Subject performance status must be Eastern Cooperative Oncology Group (ECOG) 0, 1, or 2 (see Appendix B).

F. Subject must have total bilirubin ≤ 2 mg/dL and AST and ALT ≤ 2.5 times the upper limit of normal.

G. Subject must have serum creatinine < 2 mg/dL.

H. Females of child-bearing potential (*i.e.*, women who are pre-menopausal or not surgically sterile) may participate, provided they meet the following conditions:

- Must agree to use physician-approved contraceptive methods throughout the study and for 6 months following the last dose of ciprofloxacin and/or etoposide. Adequate forms of contraception are abstinence, double-barrier methods (*e.g.*, condoms with spermicidal jelly or foam and diaphragm with spermicidal jelly or foam), oral, depot, or injectable contraceptives, intrauterine devices, and tubal ligation.

- Must have a negative serum pregnancy test within 7 days prior to beginning treatment on this study.

I. Males with female partners of child-bearing potential must agree to use physician-approved contraceptive methods (*e.g.*, abstinence, condoms, vasectomy) throughout the study and should avoid conceiving children for 6 months following the last dose of ciprofloxacin and/or etoposide.

J. Subjects must have provided written informed consent and be willing to comply with all study-related procedures.

3.3 Exclusion Criteria

Subjects with any of the following will not be eligible for study participation:

A. History of allergic or significant adverse reaction (*e.g.*, anaphylaxis, prolonged QTc, or severe tendonitis) to ciprofloxacin or etoposide.

B. Acute promyelocytic leukemia (APL) with t(15;17).

C. Prolonged baseline QTc, defined as QTc interval > 470 msec in women and > 450 msec in men, or > 480 msec in subjects with a bundle branch block.

D. Uncontrolled, clinically significant infection. Subjects with fever (temperature $\geq$ 38.3) thought to be related to leukemia are eligible assuming that blood cultures are negative during the 7 days prior to Cycle 1 Day 1 and there is no clinical evidence of active infection (*e.g.*, negative or stable radiographs and negative physical examination).

E. Ongoing, symptomatic *Clostridium difficile* infection. Subjects who are asymptomatic with negative stool for C. difficile may participate.

F. Pregnant and/or nursing.

G. History of Myasthenia Gravis.

H. Treatment with any anticancer therapy (standard or investigational) within 14 days prior to the first dose of ciprofloxacin or less than full recovery ($\leq$ CTCAE grade 1) from the clinically significant toxic effects of that treatment. The use of hydroxyurea is allowed only during the first 14 days of Cycle 1.

4. STUDY TREATMENT

All subjects entering the screening phase will receive a unique subject number. This number will be used to identify the subject throughout the study. Subjects withdrawn from the study will retain their subject number.

4.1 Etoposide and Ciprofloxacin

Ciprofloxacin will be administered orally twice daily on Days 1 to 10 of each 28-day cycle. Whenever possible, doses should be taken at 0800 hours and 2000 hours. If for some reason the doses are not taken at these times, ciprofloxacin can be taken as late as 6 hours after the scheduled time. If > 6 hours elapses, that dose of ciprofloxacin will be held and not made up. The starting dose will be 750 mg (dose level 0) with escalation to a maximum of 1000 mg (dose level +1) or de-escalation to a minimum of 500 mg (dose level -1).

Etoposide at a fixed dose of 200 mg will be administered orally once daily on Days 2 to 8 of each 28-day cycle. Subjects will be instructed to take etoposide with their morning dose of ciprofloxacin at 0800 hours on an empty stomach. If for some reason the dose is not taken at this time, it may be taken as late as 6 hours after the scheduled time. If >6 hours elapses, that dose of etoposide will be held and not made up.

A medication diary will be given to all subjects and they will be instructed to record the actual times they take both etoposide and ciprofloxacin. Doses missed will be recorded as such. Subjects will be told to call within 12 hours of missing a dose. Subjects will be instructed to bring the diary and any unused medication with them to all study visits. Any unused medication will be disposed of per institutional standard practice.

Subjects in Cycle 1 of Phase Ib who miss any etoposide or ciprofloxacin doses will be considered evaluable only if a DLT occurs. Otherwise they will be removed from the study on Day 28 and replaced in the cohort in which they were treated. Subjects in Phase Ib beyond the first cycle and those in Phase II will be considered evaluable for response if they have received at least one cycle in accord with the protocol. For these subjects non-compliance with dosing will be dealt with at the discretion of the PI.

Standard administration instructions will be given to each subject for ciprofloxacin (*e.g.*, separating administration from dairy products, calcium-fortified juices, sucralfate, antacids, or other medications that contain aluminum, magnesium, calcium, iron, or zinc). Subjects will be advised not to consume grapefruit or grapefruit juice within 7 days prior to starting ciprofloxacin and until 7 days after completion of all doses of ciprofloxacin on study.

4.2 Treatment Duration

Phase Ib subjects who developed a DLT with the first cycle will be taken off study at the Cycle 1 Day 28 visit.

For all others, treatment with ciprofloxacin and etoposide will be repeated in 28-day cycles until:

- In Cycle 2, if the treating physician decides that the subject has rapidly progressive disease despite treatment;
- Subject withdrawal of consent for continued participation in the study;
- Inter-current illness that prevents further administration of study treatment;
- Unacceptable toxicity attributed to study treatment with ciprofloxacin and etoposide;
- Completion of 2 cycles of therapy from Day 1, Cycle 1;
- Disease progression defined by >25% increase in marrow blasts (Appendix A);
- If, in the opinion of the PI or treating physician, continuation in the study could be detrimental to the subject's well-being;
- Significant deviation from inclusion/exclusion criteria in the opinion of the PI;
- Lost to follow-up (discontinuation date will be recorded as the date of last contact with subject); and/or
- Termination of the study.

Subjects with an ANC < 500/µL at the Day 28 assessment will be treated unless the Day 28 bone marrow shows < 5% cellularity. In that event, treatment will be held and a CBC will be repeated weekly until Day 42. A bone marrow will be repeated no later than Day 42 for subjects who

have treatment held. In the event that the subject does not show bone marrow recovery with a cellularity > 5% by Day 42 of treatment, the subject will be withdrawn from the study.

Table 3. Dosing Modifications

Event	Action
Disease Progression	Stop Therapy
ANC > 500/ µL, No progression	Resume Therapy
ANC < 500/ µL, Cellularity > 5%, No progression	Resume Therapy

4.3 Supportive Care

Supportive care will be administered to all study subjects per institutional guidelines.

Subjects should be prescribed and counseled to take an anti-emetic 30 – 60 minutes prior to all etoposide doses. Subjects should be counseled to take etoposide capsules on an empty stomach.

Subjects should be counseled on the expected side effects of ciprofloxacin and etoposide, and what to do if/when these occur.

4.4 Management of Toxicity

The National Cancer Institute (NCI) Common Toxicity Criteria for Adverse Events version 4 (CTCAE) will be used to grade toxicity (http://ctepinfo.nih.gov/). In Cycle 1 of Phase Ib, any patients developing DLT will be permanently withdrawn from the study.

In Phase Ib and in Phase II, for subjects receiving Cycle 2 or beyond, any toxicity which delays treatment beyond day 42 of a cycle will result in permanent withdrawal of the subject from the study. In addition, toxicity which results in missing more than ½ of all doses of etoposide or ciprofloxacin will result in permanent withdrawal from the study.

The following applies to toxicity occurring in Phase Ib or Phase II except as stated:

4.4.1 Non- Hematologic Toxicity

For any Grade 3 or greater non-hematologic adverse event (AE), deemed related or possibly related to the study intervention and/or the discretion of the investigator, the subject to stop the study drugs.

Management of non-hematologic toxicities such as nausea, vomiting, mucositis, enteritis, and/or diarrhea will follow institutional guidelines.

4.4.1.1 Infection

For any severe infection, study treatment will be held until the infection is controlled. If the infection occurs during the treatment period, missed doses will not be made up.

Management of infection will follow standard institutional guidelines.

4.4.1.2 QTc Interval

Asymptomatic prolonged QTc has been reported with quinolones, although it is not very common with ciprofloxacin. If borderline increases (0.43 to 0.45 sec in men, 0.45 to 0.47 sec in

women) in QTc are noted during screening and before each new cycle, the following are recommended:

- Maintain serum potassium > 4.0 mmol/L and serum magnesium > 2.0 mg/dL whenever possible; and
- Medications with the potential for QTc prolongation should be avoided when possible while on ciprofloxacin.

12-lead ECG recordings will be obtained during screening, on Cycle 1 Day 8, Cycle 1 Day 28, and when clinically indicated.

CTCAE V4 Grade 3 toxicity criteria for the QTc interval is ≥ 501 msec on at least two separate ECGs (Fredericia correction). If this occurs, the subject will be placed on a cardiac monitor and cardiology consultation will be obtained for confirmation. If confirmed, potassium and magnesium supplementation will be given as above. For subjects in Cycle 1 of Phase Ib, this event represents a DLT and the subject will be permanently removed from the study. For subjects beyond Cycle 1 of Phase Ib and for those in Phase II, therapy will be held until eligibility criteria are met.

4.4.2 Hematologic (Blood/Bone Marrow) Toxicity

Dose delays or dose modifications are not required for hematologic toxicities unless the disease assessment shows bone marrow hypoplasia (< 5% cellularity). It is assumed that any cytopenia at diagnosis is due to resistant AML.

Management of neutropenia, neutropenic fever, thrombocytopenia, anemia, or bleeding should follow standard guidelines within the institution.

In the absence of medical indications (surgery, bleeding) platelet transfusions should be given to maintain counts > 10,000/μL .

Erythropoietin, G-CSF, or GM-CSF may not be used during protocol treatment.

5. PRIOR AND CONCOMITANT MEDICATIONS

5.1 Prior Medications and Therapy

Relevant medical history should be obtained at screening and include prior medications and treatment history for AML, as well as prior cytotoxic therapy, chemotherapy, or radiation therapy for conditions other than AML. All medications taken within 2 weeks prior to screening, regardless of indication, should be recorded.

5.2 Concomitant Medications and Therapy

Any therapy or medication (except study drugs), administered from screening until 28 days after the last dose of either study drug, is considered a concomitant therapy or medication. However, if another course of anti-cancer therapy is initiated prior to the 28-day follow-up period visit, a record of concomitant medications will no longer be performed. If the use of any concomitant treatments (medications or procedures) becomes necessary, the treatment must be recorded, including the name of the drug or treatment, dose, route, date, indication for use, expected duration, and frequency of treatment. Assessment and documentation of concomitant medications will be done at each weekly visit.

Within 14 days of the first dose of ciprofloxacin and throughout this study, subjects may not receive any other chemotherapy or any investigational agent. The use of hydroxyurea is allowed only during the first 14 days of Cycle 1. Concomitant medications should be kept to a minimum on Days 1 – 12 of each cycle. Anti-emetics and other supportive medications such as antibiotics should be recorded on the CRF.

No other fluoroquinolone should be administered throughout the study unless mandated by resistant infection. Prophylactic levofloxacin is not allowed. If other fluoroquinolones are required, ciprofloxacin must be stopped. Otherwise, the administration of prophylactic antimicrobials is left to the discretion of the treating physician. On study Days 1 – 12 every effort should be made to give fluconazole at a dose of no more than 100 mg daily and to avoid voriconazole.

Subjects should receive appropriate treatment prior to, during and after study treatment as medically indicated to prevent tumor lysis syndrome.

Nausea and/or vomiting prophylaxis and control should be administered per institutional guidelines. Anti-emetics should be taken 15-30 minutes prior to each etoposide dose with dexamethasone 4-8 mg orally being favored. The anti-emetic chosen should be based on treatment- and subject-related factors, and prior history of control of nausea and/or vomiting. Medications that can prolong the QTc interval are relatively contraindicated on Days 1 – 12 of each cycle. A non-comprehensive list of these medications is listed in Appendix C. Subjects taking one or more of these medications may participate in the clinical study providing that they meet QTc eligibility criteria.

Medications with expected/known drug interactions with ciprofloxacin or etoposide should be avoided, if possible. Dose adjustments and/or alternative medications should be made whenever possible. Each subject's medication list should be reviewed at each study visit for any potential drug interactions, and any medication adjustment decisions should be made by the treating provider.

Ciprofloxacin is a known inhibitor of human cytochrome P450 1A2. Ciprofloxacin may increase the toxicity of medications that are metabolized by this enzyme including theophylline, tizantidine, duloxetine, propranolol, and caffeine. Therapy with these agents should be closely monitored.

Etoposide is a major substrate of human cytochrome P450 3A4 and P-glycoprotein. Medications that are strong inhibitors of this enzyme/transporter should be avoided if possible, or dose adjustments made. All subjects should be counseled to avoid grapefruit or juices containing grapefruit while on treatment with ciprofloxacin and etoposide.

The following drugs are also excluded:

- filgrastim (G-CSF, Neupogen);
- sargramostim (GM-CSF, Leukine);
- peg-filgrastim (Neulasta);
- erythropoietin (EPO, Procrit);
- darbopoietin (Aranesp);
- concomitant anti-cancer treatment(s);
- revlimid (lenalidomide);
- retinoids;

- interleukin-11;
- thalidomide;
- interferon;
- azacitidine (Vidaza);
- decitabine (Dacogen); and
- Other investigational drugs.

6. STUDY PROCEDURES

Please refer to the Study Events Schedule (Table 1).

6.1 Screening Procedures

Written informed consent must be obtained prior to performing any study-specific evaluations or tests. Tests or evaluations performed as standard of care within the specified screening period, but prior to informed consent, may be accepted for this study and need not be repeated.

The following screening assessments are required within 21 days prior to Cycle 1 Day 1 (C1D1), unless otherwise specified:

- Demographic data, including date of birth, gender, race, and smoking status (smoker or nonsmoker);
- Medical history, including major diseases and/or surgeries;
- Baseline signs and symptoms;
- Document all medications taken during the 3weeks prior to screening;
- Physical examination;
- Weight;
- Bone marrow aspirate and/or biopsy for clinical classification, flow cytometry, cytogenetic classification, and identification of commonly tested mutations in AML, (within 42 days of start of treatment (C1D1);
- ECOG performance status (see Appendix B: ECOG Performance Status);
- 12-lead electrocardiogram (ECG), including rhythm, heart rate, and PR, QRS, and QTc intervals;
- Vital signs, including systolic and diastolic blood pressure, pulse rate, respiratory rate, and temperature;
- Hematology laboratory evaluations:
    - Complete blood count (CBC) including hemoglobin, hematocrit, white blood cell (WBC) absolute differential including circulating blasts, and platelet count;
- Blood chemistry laboratory evaluations:
    - BUN, creatinine, uric acid, total bilirubin, AST, ALT, alkaline phosphatase, LDH, sodium, potassium, calcium, magnesium, phosphorous, bicarbonate, chloride, glucose, albumin, and total protein;
- Serum pregnancy test within 7 days of Cycle 1 Day 1 for females of childbearing potential; and
- Adverse event (AE) assessment (starting when the subject provides written informed consent).

6.2 On-Study Evaluations

Cycle 1 Day 1

All subjects will have the following procedures completed on the Cycle 1 Day 1 visit, unless otherwise specified:

- Document concomitant medications and transfusions;
- AE assessment;
- Physical exam;
- Vital signs, including blood pressure, pulse, respirations, and temperature;
- Weight;
- ECOG Performance Score;
- Hematology laboratory evaluations (unless performed in the 24 hours prior to visit):
    - Complete blood count (CBC) including hemoglobin, hematocrit, white blood cell (WBC) absolute differential including circulating blasts, and platelet count; and
- Blood chemistry laboratory evaluations (unless performed in the 24 hours prior to visit):
    - BUN, creatinine, uric acid, total bilirubin, AST, ALT, alkaline phosphatase, LDH, sodium, potassium, calcium, magnesium, phosphorous, bicarbonate, chloride, glucose, albumin, and total protein.

Cycle 1 Day 8

All subjects will have the following procedures:

- Document concomitant medications and transfusions;
- AE assessment;
- Physical exam;
- 12-lead electrocardiogram (ECG), including rhythm, heart rate, and PR, QRS, and QTc intervals;
- Vital signs, including blood pressure, pulse, respirations, and temperature;
- Weight;
- ECOG Performance Score;
- Hematology laboratory evaluations (unless performed in the 24 hours prior to visit):
    - Complete blood count (CBC) including hemoglobin, hematocrit, white blood cell (WBC) absolute differential including circulating blasts and platelet count;
- Blood chemistry laboratory evaluations (unless performed in the 24 hours prior to visit):
    - BUN, creatinine, uric acid, total bilirubin, AST, ALT, alkaline phosphatase, LDH, sodium, potassium, calcium, magnesium, phosphorous, bicarbonate, chloride, glucose, albumin, and total protein; and

Cycle 1 Days 15 and 22

All subjects will have the following procedures:

- Document concomitant medications and transfusions;
- AE assessment;
- Physical exam;
- Vital signs, including blood pressure, pulse, respirations, and temperature;
- Weight;
- ECOG Performance Score;

- Hematology laboratory evaluations (unless performed in the 24 hours prior to visit):
    - Complete blood count (CBC) including hemoglobin, hematocrit, white blood cell (WBC) absolute differential including circulating blasts and platelet count; and
- Blood chemistry laboratory evaluations (unless performed in the 24 hours prior to visit):
    - BUN, creatinine, uric acid, total bilirubin, AST, ALT, alkaline phosphatase, LDH, sodium, potassium, calcium, magnesium, phosphorous, bicarbonate, chloride, glucose, albumin, and total protein.

Cycle 1 Day 28

The C1D28 visit may occur ± 48 hours from the protocol-specified date. All subjects will have the following procedures:

- Document concomitant medications and transfusions;
- AE assessment;
- Physical exam;
- Vital signs, including blood pressure, pulse, respirations, and temperature;
- Weight;
- 12-lead electrocardiogram (ECG), including rhythm, heart rate, and PR, QRS, and QTc intervals;
- ECOG Performance Score;
- Hematology laboratory evaluations:
    - Complete blood count (CBC) including hemoglobin, hematocrit, white blood cell (WBC) absolute differential including circulating blasts, and platelet count;
- Blood chemistry laboratory evaluations:
    - BUN, creatinine, uric acid, total bilirubin, AST, ALT, alkaline phosphatase, LDH, sodium, potassium, calcium, magnesium, phosphorous, bicarbonate, chloride, glucose, albumin, and total protein;
- Bone marrow aspirate and/or biopsy for clinical classification (cellularity, blast assessment) andcytogenetic testing, ; and
- Serum or urine pregnancy test – if applicable for females of childbearing potential.

At C1 D28, the subject will have completed protocol-specified treatment for Cycle 1. For subjects in Phase Ib, DLT assessment will occur at this point.

Cycle 2/Days 1, 8, 15, and 22

All subjects will have the following procedures completed on the visit, unless otherwise specified:

- Document concomitant medications and transfusions;
- AE assessment;
- Physical exam;
- Vital signs, including blood pressure, pulse, respirations, and temperature;
- Weight;
- ECOG Performance Score;
- Hematology laboratory evaluations (unless performed in the 24 hours prior to visit):
    - Complete blood count (CBC) including hemoglobin, hematocrit, white blood cell (WBC) absolute differential including circulating blasts, and platelet count; and

- Blood chemistry laboratory evaluations (unless performed in the 24 hours prior to visit):
    - BUN, creatinine, uric acid, total bilirubin, AST, ALT, alkaline phosphatase, LDH, sodium, potassium, calcium, magnesium, phosphorous, bicarbonate, chloride, glucose, albumin, and total protein.

Cycle 2 /Day 28 (±2) and EOT (± 7)

The Day 28 visit may occur ± 48 hours and EOT may occur within 7 days from the protocol-specified date. All subjects will have the following procedures:

- Document concomitant medications and transfusions;
- AE assessment;
- Physical exam;
- Vital signs, including blood pressure, pulse, respirations, and temperature;
- Weight;
- ECOG Performance Score;
- Hematology laboratory evaluations:
    - Complete blood count (CBC) including hemoglobin, hematocrit, white blood cell (WBC) absolute differential including circulating blasts, and platelet count;
- Blood chemistry laboratory evaluations:
    - BUN, creatinine, uric acid, total bilirubin, AST, ALT, alkaline phosphatase, LDH, sodium, potassium, calcium, magnesium, phosphorous, bicarbonate, chloride, glucose, albumin, and total protein;
- Bone marrow aspirate and/or biopsy for clinical classification (cellularity, blast assessment) and cytogenetic testing,; and
- Serum or urine pregnancy test – if applicable for females of childbearing potential
- 12-lead electrocardiogram (ECG), including rhythm, heart rate, and PR, QRS, and QTc intervals (only at EOT)

If a subject achieves CR, or at end of treatment bone marrow assessments will be performed as clinically indicated.

6.3 Treatment Discontinuation

The end of treatment visit will occur at the C1 D28 visit in subjects with progressive disease or those in Phase Ib who develop DLT or at study withdrawal, even if the date does not correspond to a protocol-specified visits. Other reasons for withdrawal of treatment are given in Section 4.2. For subjects completing study stipulated two cycles of treatment (phase II portion of the study) will complete end of treatment assessment as described in section 7.2

6.4 Follow up/Survival

Subjects will have a follow-up visit for the evaluation of adverse events and concomitant medications 28 days (± 7 days) after the last study medication dose.

Follow-up evaluations will be performed for clinically significant abnormal physical examinations or abnormal laboratory findings, 12-lead ECGs, or vital signs, as deemed necessary by the treating physician.. All subjects who have adverse events, whether considered associated

with the use of the study drugs or not, must be monitored to determine the outcome. The clinical course of the adverse event will be followed according to accepted standards of medical practice, even after the end of the period of observation, until a satisfactory explanation is found or the Principal Investigator deems it medically justifiable to terminate follow-up.

All subjects will be followed monthly until: disease progression, death, or until 3 years from the on-study date.

7. DATA MEASUREMENTS AND METHODS OF COLLECTION

7.1 Data Measurements

7.1.1 Safety Data

Safety variables for this study include the following:

- DLTs;
- Physical examinations;
- Vital signs;
- 12-lead ECGs;
- Clinical laboratory measurements, including hematology and serum chemistry; and
- Adverse events.

7.1.2 Efficacy Data

The efficacy outcomes of interest will be CR (including CRi and CRp) as defined in Appendix A. The efficacy variables, which will be used to assess response, include hemoglobin, ANC, platelets, circulating blasts, bone marrow aspirate and biopsy.

7.2 Collection Methods

7.2.1 Bone Marrow Samples

Bone marrow aspiration and biopsy will be collected using standard institutional procedures at the following time points:

- Screening: within 42 days prior to Cycle 1 Day 1;
- Day 28 (± 7 days) after each cycle until CR or progressive disease

Bone marrow aspirations (6 to 8 mL) and core biopsies will be collected as per standard of care for leukemia treatment. As per standard of care, these specimens will also be sent for flow cytometry. Routine cytogenetics, FISH and genetic analysis will be obtained if abnormalities were present at the time of study entry.

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7.2.2 Safety Measurements

Safety measurements for this study include AEs, clinical laboratory measurements (hematology and serum chemistry), physical examination findings, vital signs, and 12-lead ECGs.

7.2.3 Adverse Events

Adverse events that occur prior to dosing will be collected on Day 1 by questioning the subject regarding their occurrence since the screening visit. Adverse events will be collected at each

clinic visit based on observation and spontaneous reporting.

7.2.4 Clinical laboratory assessments

Clinical laboratory assessments are outlined in Table 1.

Hematology assessments will be performed during the Screening period and on Days 1, 8, 15, 22, and 28 of each cycle. The hematology assessments will include:

- Complete blood count (CBC) including hemoglobin, hematocrit, white blood cell (WBC) absolute differential including circulating blasts, and platelet count.

Blood chemistry assessments will be performed during the Screening period on Days 1, 8, 15, 22, and 28 of each cycle. The chemistry assessments will include:

- Electrolytes: (sodium, potassium, calcium, magnesium, phosphorous, bicarbonate, and chloride);
- Renal function: (BUN, creatinine, and uric acid);
- Liver function: SGOT (also known as AST), SGPT (also known as ALT), total bilirubin;
- LDH, and alkaline phosphatase; and
- Other: (glucose, albumin, and total protein).

Serum pregnancy tests for females of childbearing potential will be performed during the screening period and on day 28 of each cycle. If delayed, a pregnancy test will be repeated within 7 days of the first day of the next cycle.

Approximately 50 mL of blood will be collected for safety assessments from each subject over the course of each 28-day cycle.

Abnormal, clinically significant results as assessed by the Principal Investigator should be repeated to rule out laboratory error. Persistent relevant abnormal changes from baseline must be followed up until the cause is determined or until they return to the premedication value. Hematology and serum chemistry will be carried out according to the standard operating procedures by the validated local laboratory.

7.2.5 Physical Examination, Vital Signs and ECG

A physical examination and vital signs (systolic and diastolic blood pressure, pulse rate, respiratory rate, and temperature) will be conducted during the Screening period and on each study visit (Days 1, 8, 15, 22, and 28) of every cycle of CE. A 12-lead ECG (rhythm, heart rate, and PR, QRS, and QTc intervals) will be performed during the Screening period, on Cycle 1 Day 8, Cycle 1 Day 28, and as clinically indicated.

8. ADVERSE EVENTS

8.1 Definitions

8.1.1 Adverse Event

The term “adverse event” includes any sign, symptom, syndrome, or illness that appears or worsens in a subject during the period of observation in the clinical study and that may impair the wellbeing of the subject. The term also covers laboratory findings or results of other diagnostic procedures that are considered to be clinically significant (*e.g.*, that require unscheduled diagnostic procedures or treatment measures, or result in withdrawal from the study).

The adverse event may be:

- A new illness/condition;
- Worsening of a sign or symptom of the condition under treatment, or of a concomitant illness/condition;
- An effect of the study drug; or
- A combination of 2 or more of these factors.

No causal relationship with the study drug or with the clinical study itself is implied by the use of the term "adverse event."

Surgical procedures themselves are not adverse events; they are therapeutic measures for conditions that require surgery. The condition(s) for which the surgery is required may be an adverse event. Planned surgical measures permitted by the clinical study protocol and the condition(s) leading to these measures are not adverse events.

When a clear diagnosis is available that explains the abnormal objective findings, this diagnosis will be recorded as an adverse event and not the abnormal objective findings (*e.g.*, viral hepatitis will be recorded as the adverse event and not the transaminase elevation). If a definitive diagnosis is not available, then the sign(s) (*e.g.*, clinically significant elevation of transaminase levels) or symptom(s) (*e.g.*, abdominal pain) will be recorded as the adverse event.

Adverse events fall into the categories "serious" and "non-serious."

8.1.2 Serious Adverse Event

A serious adverse event is one that at any dose of the study drug or at any time during the period of observation:

- Results in death;
- Is life-threatening;
- Requires inpatient hospitalization or prolongation of existing hospitalization;
- Results in persistent or significant disability/incapacity;
- Is a congenital anomaly/birth defect; or
- Is medically important.

Life-threatening means the subject was at immediate risk of death at the time of the serious adverse event; it does not refer to a serious adverse event that hypothetically might have caused death if it were more severe.

Persistent or significant disability or incapacity means there is a substantial disruption of a person's ability to carry out normal life functions.

Medical and scientific judgment should be exercised in deciding whether other adverse events may be considered serious because they jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above. Examples of such events are: intensive treatment in an emergency room or at home for allergic bronchospasm, blood dyscrasias, or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse. "Medically important" should be marked only if no other serious criteria are met.

An "unexpected SAE" is any SAE for which the nature, specificity or severity is not consistent with the currently known adverse reaction to ciprofloxacin or etoposide

Clarification of the difference in meaning between "severe" and "serious"

The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as severe headache). Any grade ≥ 3 adverse event per CTCAE is generally considered severe AE. This is not the same as "serious," which is based on the outcome or action criteria usually associated with events that pose a threat to life or functioning. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

8.1.3 Non-Serious Adverse Event

A non-serious adverse event is any adverse event not meeting any of the serious adverse event criteria.

8.2 Period of Observation

For the purpose of this study, the period of observation for adverse events starts on the day the subject signs informed consent. For subjects who fail screening, the period of observation ends on the date of screening failure. Treated subjects, including those who were prematurely discontinued from the study, will be followed for any adverse events that occur during the study from the time the subject signs the informed consent form until 28 days (± 7 days) following the last dose of study treatment (i.e., the Follow-up Visit). However, if another course of anti-cancer therapy is initiated prior to the 28-day follow-up period visit, collection of adverse events will no longer be performed, with the exception of events that may be possibly, probably, or definitely related to the investigational agent or are clinically significant.

8.3 Documenting and Reporting of Adverse Events by Investigator

All adverse events that occur after the subject has signed informed consent must be documented on the pages provided in the case report form. The following approach will be taken for documentation:

- All adverse events (whether serious or non-serious) must be documented on the "Adverse Event" page of the case report form.
- For adverse events classified as serious (see Section 8.4.1) the Investigator must report the event within 24 hours to UFHCC PMO. All SAE's must also be reported to the IRB and DISC in accordance with the institution's policy.

Every attempt should be made to describe the adverse event in terms of a diagnosis that encompasses the component signs and symptoms. If only nonspecific signs or symptoms are present, then these should be recorded as separate diagnoses on the pages of the case report form.

All subjects who have adverse events, whether considered associated with the use of study drug or not, must be monitored to determine the outcome. The clinical course of the adverse event will be followed according to accepted standards of medical practice, even after the end of the period of observation, until a satisfactory explanation is found or the Principal Investigator considers it medically justifiable to terminate follow-up. Should the adverse event result in death, a full pathologist's report should be supplied, if possible.

8.3.1 Assessment of Causal Relationship of Study Drug

The Investigator will provide an assessment of the potential causal relationship between adverse

events and study medication by determining whether or not there is a reasonable possibility that the event was caused by the study medication. The relationship or association of the adverse event to the study medication will be characterized as not related, probably not related, possibly related, probably related, or related:

Not Related: There is not a temporal relationship to the study drug administration or the adverse event is clearly due only to the progression of the underlying disease state, intercurrent illness, concomitant medication, concurrent therapy or other known cause.

Probably Not Related: There is little or no chance that the study drug administration caused the adverse event; the event is most likely due to another competing cause, including intercurrent illness, progression or expression of the disease state, or a reaction to a concomitant medication or concurrent therapy appearing to explain the reported adverse event.

Possibly Related: The association of the adverse event with the study drug administration is unknown; however, the adverse event is not reasonably attributed to any other condition.

Probably Related: When a reasonable temporal relationship exists between the adverse event and the study drug administration; significant symptoms abate upon discontinuation of the study drug and there is a reasonable explanation based on known characteristics of the study drug and there is no clear association with preexisting disease or therapy, intercurrent illness, concurrent therapy or other factor(s).

Related: When the adverse event is a known side effect of the study drug or there is a temporal relationship to the administration of the study drug; or the adverse event reappears upon re-administration of the study drug (rechallenge); or the significant symptoms of the adverse event abate upon discontinuation of the study drug (dechallenge).

8.3.2 Intensity of Adverse Events

The intensity of adverse changes in physical signs or symptoms will be graded according to the CTCAE version 4. For all other adverse events not described in the CTCAE, the intensity will be assessed by the Investigator using the following categories:

Mild (Grade 1) – transient or mild discomfort; no limitation in activity; no medical intervention/therapy required.

Moderate (Grade 2) – mild to moderate limitation in activity, some assistance may be needed; no or minimal medical intervention/therapy required.

Severe (Grade 3) – marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalization is possible.

Life-threatening (Grade 4) – extreme limitation in activity, significant assistance required; significant medical intervention/therapy required, hospitalization or hospice care probable.

Death (Grade 5) – the event resulted in death.

8.3.3 Action Taken with Study Drug

The action the Investigator took with study drug as a result of the event should be recorded as one of the following:

None – No action was taken with regard to the study drug as a result of the adverse event.

Interrupted – Study drug was stopped due to the adverse event, but was later resumed at the

same dose.

Dose decreased – The dose of study drug was decreased as a result of the adverse event.

Permanently discontinued – The subject was withdrawn from the study due to the adverse event.

Only one item should be chosen. If multiple actions apply, the following "worst case" scenario hierarchy should be used to determine the preferred entry:

Discontinued > dose decreased > therapy interrupted.

8.3.4 Definition of Outcome

The outcome of the AE should be recorded as one of the following:

Resolved without sequelae – The subject fully recovered from the adverse event with no observable residual effects.

Resolved with sequelae – The subject recovered from the adverse event with observable residual effects.

Not resolved – The adverse event was present at the time of last observation.

Death – The subject died as a result of the adverse event.

8.4 Immediately Reportable Events

8.4.1 Serious Adverse Events

Serious adverse events (SAEs) must be reported to the UFHCC Project Management Office (pmo@cancer.ufl.edu) within 24 hours of becoming aware of the event, and reported to the UF Health DISC within 5 days of being made aware of the event. All SAE's must also be reported to the IRB in accordance with the institution's policy per 21 CFR part 56.

The initial report must be as complete as possible, including details of the current illness and SAE and an assessment of the causal relationship between the event and study drug. Information not available at the time of the initial report (for example, an end date for the adverse event or laboratory values received after the report) must be documented and provided to DISC,PMO, and IRB in accordance with their reporting guidelines.

8.4.2 Other Events Requiring Immediate Reporting

Any overdose of treatment should be reported to the UF Health DISC within 5 days of being made aware of the event, and UFHCC Project Management Office (pmo@cancer.ufl.edu) within 24 hours of becoming aware of the event, regardless of association with a separate adverse event. In case the overdose did not result in any adverse event, the Investigator should report this as "overdose, no adverse event" and provide the intended amount, as well as the actual amount, of drug administered. In the event of overdose or exaggerated response, appropriate supportive measures should be employed. Actual treatment should depend on the severity of the clinical situation and the judgment and experience of the treating physician.

9. DISCONTINUATION

9.1 Discontinuation of Subjects

A subject must be discontinued from protocol-prescribed therapy under the circumstances defined in Section 4.2.

If a female subject or the female partner of a male subject who is required to use defined contraceptive methods becomes pregnant after the study, the female subject or pregnant partner must be followed until the outcome of the pregnancy is known.

As far as possible, all end-of-study examinations must be performed on all subjects who receive the study drug but do not complete the study according to protocol. All subjects who discontinue from protocol-prescribed therapy for any of the reasons above will be followed for at least 28 days following the last day of study drug administration.

9.2 Replacement of Subjects

Subjects who are withdrawn prior to C1D1 for any reason will be replaced.

Phase Ib - Subjects who withdraw or are withdrawn from study prior to C1 Day 28 without a DLT will be replaced by another subject.

Phase II - Subjects who withdraw or are withdrawn prior to receiving one full cycle of therapy will be replaced by another subject.

9.3 Study Stopping Rules

During Phase Ib the study will be stopped if Dose Level -1 is not tolerated due to excessive toxicity.

During Phase II, the stopping rule for efficacy is inherently incorporated by using the Simon two stage design. For safety stopping rule, if 35% of the stage I study subjects (i.e., 5 subjects= 14 x 0.35) experience adverse events, the study will not proceed to the second stage of the study and will be terminated, regardless of efficacy result.

Also, as a Level 3 - high risk study as defined by DISC committee the study is subject to semi-annual review of the safety data. A decision may be made to allow additional subjects to be enrolled and treated after consultation with the DISC.

10. DATA AND SAFETY MONITORING

This protocol will be reviewed and monitored by the University of Florida Health Cancer Center (UFHCC) Data Integrity and Safety Committee (DISC). This study will have, at a minimum, semi-annual monitoring by the UFHCC DISC. Any adverse event fulfilling expedited reporting requirements must be reported to the DISC coordinator within 5 working days. The DISC coordinator will forward the report to the DISC Chairperson. Questions about subject safety or protocol performance will be addressed with the sponsor-investigator, statistician and study team members. Should any major concerns arise, DISC will offer recommendations regarding whether or not to suspend the trial.

UFHCC DISC data and safety monitoring activities include:

- Review of clinical trial conducted for progress and safety
- Review of all adverse events requiring expedited reporting as defined in the protocol
- Review of reports generated by data quality control review process
- Notification of the sponsor-investigator of recommended action
- Notification of sites coordinated by the UFHCC of adverse events requiring expedited reporting and subsequent committee recommendations for study modifications

In compliance with the UFHCC data and safety monitoring plan, the PI will provide a Data Integrity and Safety Committee Report to DISC at the predetermined timelines for the level of risk category assigned during the initial SRMC review.

UFHCC investigator-initiated protocols will be classified into one of the following categories of risk:

Level 1 – Low risk non-therapeutic interventional trials.
Level 2 – Moderate risk therapeutic (i.e., drug, biologic, or device) trials using FDA approved or commercially available compounds or interventions.
Level 3 – High risk therapeutic trials (i.e., investigator-sponsored INDs, Phase I trials, studies requiring biosafety approval, or other areas that may be designated by NIH as high risk).
Level 4 – Complex trials involving very high risk to subjects and a high level complexity (i.e., first in human or gene transfer studies).

The PI will summarize and provide DISC with all pertinent data related to the level of risk assigned by SRMC. This protocol summary will include a minimum of the following:

- The UF IRB assigned protocol number, UFHCC assigned protocol number, protocol title, PI name, data coordinator name or primary study coordinator, regulatory coordinator name, and statistician.
- Date of initial UF IRB approval, date of most recent consent UF IRB approval/revision, date of UF IRB expiration, study status, and phase of the study.
- Study target accrual and study actual accrual.
- Protocol objectives with supporting data and list of number of study participants who have met each objective.
- Measures of efficacy.
- Early stopping rules with supporting data and a list of the number of study participants who have met the early stopping rules.
- Summary of toxicities and protocol deviations.
- Summary of any recent literature which may affect the safety or ethics of the trial.

As part of the responsibilities assumed by conducting this study, the Principal Investigator (PI) agrees to maintain and have available for monitoring adequate case records (accurate source documents and CRFs) for the subjects treated under this protocol.

The PI will be primarily responsible for monitoring of adverse events, protocol violations, and other immediate protocol issues. The study coordinator will collect information on subjects enrolled through the use of electronic or paper adverse event (AE) forms, CRFs, and Informed Consent forms.

Identification of oversight responsibility:

The PI has primary responsibility.

The UFHCC DISC will meet at least semi-annually to review accrual, patterns and frequencies of all adverse events, protocol violations, and when applicable, internal audit results.

Description of internal (PI) safety review and monitoring process:

Adverse events will be reported along with all of the other collected data in the OnCore database. The PI or PI designee will report all adverse events to the UFHCC DISC and IRB per reporting guidelines.

11. STATISTICAL METHODS

The sections below provide an overview of the statistical considerations and analyses.

11.1 Determinations of Sample Size

An estimate of 8-24 patients are to be enrolled to Phase Ib portion of the study accounting for a 25% drop-out rate. Precise sample size cannot be defined, as it is dependent on the observed toxicity rate in this phase.

Subjects will be enrolled in the Phase II portion of the study once the MTD has been determined. The primary intention is to determine the complete response rate (CR + CRi, see Appendix A) of ciprofloxacin and etoposide given at the MTD to subjects with resistant AML, which is achieved at the end of any treating cycle. The CR rate for AML subjects receiving etoposide alone is approximately 20% (the null hypothesis). We expect that the combination of ciprofloxacin and etoposide will improve the CR rate to 40%. In order to detect this difference with a type I error rate of 0.10 and power of 80%, when the true response rate is 40%, a minimum of 24 subjects who are evaluable for response will need to be accrued, based on the Simon's two-stage Minimax design. Specifically, in the first stage, we will enroll 14 evaluable subjects to this phase. The accrual will be temporarily suspended once the 14th evaluable subject is enrolled, pending response evaluation. If 2 or fewer respond, in these 14 subjects, the study will be stopped. Otherwise, continue to accrue additional 10 evaluable subjects (for a total of 24 evaluable subjects)., The null hypothesis of 20% CR will be rejected if 8 or more CRs are observed in 24 subjects. Assuming 20% drop out rate, the total number of subjects to be enrolled to the Phase II portion is 30

11.2 Analysis Populations

11.2.1 Safety Population

The safety population will consist of all subjects who received at least one dose of each study drug and had at least one post-dose safety assessment. Safety summaries will include all subjects in the safety population.

11.2.2 Dose Limiting Toxicity Evaluable Population (Phase Ib)

Subjects withdrawn before C1D1 will not be evaluable for dose escalation decisions and will be replaced. A subject is evaluable for dose-escalation decisions provided: they receive all drug doses and remain on study until evaluation at Cycle 1 Day 28 without a DLT; or they experienced a DLT during Cycle 1. Subjects who miss any etoposide or ciprofloxacin doses or

withdraw from study without a DLT and prior to Cycle 1 Day 28 are not evaluable and will be replaced in their cohort of treatment. Tabulations of DLTs will only include the DLT-evaluable population.

11.2.3 Efficacy Evaluable Population

The efficacy population will include all subjects who receive at least 1 cycle of ciprofloxacin and etoposide and had at least one post-dose efficacy assessment.

11.3 Analysis Methods

11.3.1 Safety

Safety evaluation will include monitoring for adverse events, scheduled laboratory assessments, vital sign measurements, ECGs, and physical examinations. The intensity of adverse changes in physical signs or symptoms will be graded according to the CTCAE version 4. For all other adverse events not listed in the CTCAE, the intensity of events will be assessed by the PI using a 5-point scale as described in Section 8.3.2.

The number of adverse events and the incidence of adverse events will be summarized. Adverse events will be summarized by maximum intensity (as described in Section 8.3.2) and relationship to study drug for each treatment group. Separate summaries will be provided for all adverse events, serious adverse events, treatment-related adverse events, and other significant adverse events (*e.g.*, adverse events leading to study discontinuation).

Clinical laboratory results will be listed by subject or, as appropriate, summarized descriptively by treatment group, which will include a display of change from baseline. Laboratory values outside of the normal ranges will be identified. Clinically significant hematologic laboratory abnormalities (*i.e.*, meet Grade 3, 4, or 5 criteria according to CTCAE) will be listed and summarized.

Physical examination, vital sign, and ECG data will be listed for each subject at each visit. If appropriate, descriptive statistics for vital signs, both observed values and changes from baseline, will be summarized by treatment group.

11.3.2 Efficacy

All efficacy endpoints will be summarized descriptively using frequency distributions. In addition, 95% confidence intervals will be provided for the proportion of each response/improvement.

12. EMERGENCY PROCEDURES

12.1 Emergency Contact

In emergency situations, the treating physician should contact the Principal Investigator by telephone at the number listed on the title page of the protocol.

12.2 Emergency Identification of Investigational Products

This is an open-label study; therefore, study drug will be identified on the package labeling.

12.3 Emergency Treatment

During and following a subject's participation in the study, the treating physician and/or institution should ensure that adequate medical care is provided to a subject for any adverse events, including clinically significant laboratory values, related to the study.

13. ADMINISTRATIVE CONSIDERATIONS

13.1 Good Clinical Practice

The procedures set out in this study protocol pertaining to the conduct, evaluation, and documentation of this study are designed to ensure that the Principal Investigator and Co-Investigators abide by Good Clinical Practice (GCP), as described in International Conference on Harmonization (ICH) Guideline E6 and in accordance with the general ethical principles outlined in the Declaration of Helsinki. The study will receive approval from an Institutional Review Board (IRB) prior to commencement. The Principal Investigator will conduct all aspects of this study in accordance with applicable national, state, and local laws of the pertinent regulatory authorities.

13.2 Delegation of Investigator Responsibilities

The Principal Investigator will ensure that all persons assisting with the study are adequately informed about the protocol, any amendments to the protocol, the study treatments, and their study-related duties and functions. The Principal Investigator will maintain a list of Co-Investigators and other appropriately qualified persons to whom he has delegated significant study-related duties.

13.3 Subject Information and Informed Consent

Before being enrolled in this clinical trial, the subject must consent to participate after the nature, scope, and possible consequences of the clinical study have been explained in a form understandable to him or her. An informed consent document that includes both information about the study and the consent form will be prepared and given to the subject. This document will contain all ICH, GCP, and locally required regulatory elements. The document must be in a language understandable to the subject and must specify the person who obtained informed consent.

After reading the informed consent document, the subject must give consent in writing. The written informed consent will be obtained prior to conducting any study-related procedures or tests. The subject's consent must be confirmed at the time of consent by the personally dated signature of the person conducting the informed consent discussions. A copy of the signed consent document must be given to the subject.

The PI will retain the original signed consent document. The PI will not undertake any measures specifically required only for the clinical study until valid consent has been obtained.

13.4 Confidentiality

Each subject will be assigned a unique acrostic that will contain no protected health information. The PI will retain a copy of the acrostic key in a locked office. Only the study number and unique acrostic will be recorded in the CRF, and if the patient's name appears on any other

document (*e.g.*, pathologist report), it must be redacted before a copy of the document is placed in study files. Study files stored on a computer will be stored in accordance with local data protection laws. Subjects will be told that the IRB, UF Health Data Integrity and Safety Committee, or regulatory authorities may inspect their medical records to verify the information collected, and that all personal information made available for inspection will be handled in strictest confidence and in accordance with local data protection law.

13.5 Protocol Amendments

Once the study has started, amendments should be made only in exceptional cases. The changes then become part of the study protocol.

13.6 Record Retention

Essential documents will be retained by the PI for a minimum of 3 years after study closure at IRB. Essential documents include the following:

- Signed informed consent documents for all subjects;
- Subject identification code list, screening log, and enrollment log;
- Record of all communications between the PI and the IRB;
- Composition of the IRB;
- Record of all communications between the PI and the DISC;
- Composition of the DISC;
- List of Co-Investigators and other appropriately qualified persons to whom the PI has delegated significant study-related duties, together with their roles in the study, curriculum vitae, start/stop date(s) of participation, and their signatures;
- Copies of CRFs and of documentation of corrections for all subjects (for electronic case report forms [eCRFs], electronic files of the eCRF and audit trails will be utilized and provided to the site, as necessary);
- Drug accountability records;
- Record of any body fluids or bone marrow samples retained; and
- Other source documents (subject records, hospital records, laboratory records, etc.).

14. APPENDIX

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14.1 Appendix A: AML Response Criteria According to IWG

Response Criterion	Neutrophils (µL)	Platelets (µL)	Bone Marrow Blasts (%)	Other
CR	>1,000	>100,000	<5	Transfusion independent, no evidence of EMD
CRi (CR with incomplete blood counts)	<1,000	<100,000	<5	No evidence of EMD
CRp (CR with incomplete platelet recovery)	>1,000	<100,000	<5	No evidence of EMD
Disease Progression			> 25% increase from baseline	

Key: AML=acute myelogenous leukemia; CR=complete remission; EMD=extramedullary disease; IWG=International Working Group;

14.2 Appendix B: ECOG Performance Status

ECOG Score	Description	Karnofsky Score	Description
0	Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity, minor signs or symptoms of disease.
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, (*e.g.*, light house work, office work).	80	Normal activity with effort, some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or do active 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.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable medical assistance and frequent medical care.
3	Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indications. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

Adapted from: Cheson BD, Bennett JM, Kopecky KJ, et al. Revised recommendations of the International Working Group for Diagnosis, Standardization of Response Criteria, Treatment Outcomes, and Reporting Standards for Therapeutic Trials in Acute Myeloid Leukemia. J Clin Oncol. 2003;21(24):4642-4649.

Adapted from: Center For International Blood & Marrow Transplant Research: http://www.cibmtr.org/DataManagement/TrainingReference/Manuals/DataManagement/Documents/appendix-l.pdf

14.3 Appendix C: Medications That Can Prolong QTc (Non-Comprehensive)

Generic Name	Brand Name	Class/Clinical Use
Amiodarone	Cordarone®, Pacerone®	Anti-arrhythmic / abnormal heart rhythm
Arsenic trioxide	Trisenox®	Anti-cancer / Leukemia
Astemizole	Hismanal®	Antihistamine / Allergic rhinitis
Bepridil	Vascor®	Anti-anginal / heart pain
Chloroquine	Aralen®	Anti-malarial / malaria infection
Chlorpromazine	Thorazine®	Anti-psychotic/ Anti-emetic / schizophrenia/ nausea
Cisapride	Propulsid®	GI stimulant / heartburn
Clarithromycin	Biaxin®	Antibiotic / bacterial infection
Disopyramide	Norpace®	Anti-arrhythmic / abnormal heart rhythm
Dofetilide	Tikosyn®	Anti-arrhythmic / abnormal heart rhythm
Domperidone	Motilium®	Anti-nausea / nausea
Droperidol	Inapsine®	Sedative;Anti-nausea / anesthesia adjunct, nausea
Erythromycin	E.E.S.®, Erythrocin®	Antibiotic;GI stimulant / bacterial infection; increase GI motility
Fluconazole	Diflucan ®	Anti-fungal
Halofantrine	Halfan®	Anti-malarial / malaria infection
Haloperidol	Haldol®	Anti-psychotic / schizophrenia, agitation
Ibutilide	Corvert®	Anti-arrhythmic / abnormal heart rhythm
Levomethadyl	Orlaam®	Opiate agonist / pain control, narcotic dependence
Mesoridazine	Serentil®	Anti-psychotic / schizophrenia
Methadone	Methadose®, Dolophine®	Opiate agonist / pain control, narcotic dependence
Pentamidine	Pentam®, NebuPent®	Anti-infective / pneumocystis pneumonia
Pimozide	Orap®	Anti-psychotic / Tourette's tics
Probucol	Lorelco®	Antilipemic / Hypercholesterolemia
Procainamide	Procan®, Pronestyl®	Anti-arrhythmic / abnormal heart rhythm
Quinidine	Cardioquin®, Quinaglute®	Anti-arrhythmic / abnormal heart rhythm
Sotalol	Betapace®	Anti-arrhythmic / abnormal heart rhythm
Sparfloxacin	Zagam®	Antibiotic / bacterial infection
Terfenadine	Seldane®	Antihistamine / Allergic rhinitis
Thioridazine	Mellaril®	Anti-psychotic / schizophrenia
Voriconazole	V-Fend ®	Anti-fungal

14.4 Appendix D: Statistical Characteristics of the "3+3" Design

Probabilities of Stopping or Continuing Dose Escalation for Given Probabilities of DLT Associated with the Dose Level

True Probability of DLT at a Dose Level	0.05	0.1	0.2	0.33	0.4	0.5	0.6	0.67	0.7	0.8
Probability of Dose Escalation Based on 3 or 6 Subjects (2 or more DLTs)	0.97	0.91	0.71	0.43	0.31	0.17	0.08	0.04	0.03	0.01
Probability of Ceasing Dose Escalation Based on 3 or 6 Subjects (2 or more DLTs)	0.03	0.09	0.29	0.57	0.69	0.83	0.92	0.96	0.97	0.99
Probability Based on only 3 Subjects (0 DLT)	0.86	0.73	0.51	0.30	0.22	0.13	0.06	0.04	0.03	0.01

Key: DLT = dose limiting toxicity

The probability that dose escalation will cease is at least 92% when the DLT probability is > 60%. If the true probability of DLT for a dose level is at least 33%, the probability of stopping dose escalation is at least 57%. If the true probability of DLT for a dose level is less than 10%, the probability of stopping dose escalation is less than 10%. The probability of dose escalation continuing is at least 91% when the DLT probability is < 10%.

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