TOLERO PHARMACEUTICALS
Sumitomo Dainippon Pharma Global Oncology

AMENDED CLINICAL STUDY PROTOCOL

Study Title: A Phase 2, Randomized, Biomarker-driven, Clinical Study in Patients with Relapsed or Refractory Acute Myeloid Leukemia (AML) with MCL-1 Dependence ≥***30***%

Protocol Number: TPI-ALV-201

IND Number: 057729

Study Drug/Agents: Alvocidib (formerly flavopiridol)

Phase of Development: Phase 2

Medical Monitors:

[redacted]

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Sponsor Contact:

[redacted]

Sponsor:

[redacted]

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Date of Amendment 4:	03 March 2017	***Date of Amendment 11:***	***15 August 2019***
Date of Amendment 5:	29 August 2017		
Date of Amendment 6:	06 October 2017		

PROTOCOL SIGNATURE PAGE

SPONSOR SIGNATURE

I have carefully read the protocol, TPI-ALV-201 Amendment ***11*** titled "A Phase 2, Randomized, Biomarker-driven, Clinical Study in Patients with Relapsed or Refractory Acute Myeloid Leukemia (AML) with MCL-1 Dependence ≥***30***%*"* and confirm this is the approved current version.

Sponsor's Signature	Date (DD/MMM/YYYY)
Printed Name	

INVESTIGATOR'S SIGNATURE

I have carefully read this protocol, TPI-ALV-201 Amendment ***11***, and commit to conduct the study as outlined herein, in accordance with the International Council on Harmonisation (ICH), Good Clinical Practices (GCPs) and the Declaration of Helsinki, and comply with the obligations and requirements of the Clinical Investigator and other requirements as listed in Title 21 of the United States Code of Federal Regulations (CFR) and other applicable regulations.

Investigator's Signature	Date (DD/MMM/YYYY)
Printed Name	Name of Institution/Research Facility

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ABBREVIATIONS

7&3	Refers to the regimen of Ara-c: Days 1-7 & Daunorubicin: Days 1, 2, 3
ACM	**A**lvocidib/**C**ytarabine/**M**itoxantrone (previously referred to as FLAM)
AE	Adverse event
AGC	Absolute granulocyte count
ALT	Alanine aminotransferase
AM	**A**ra-c/**M**itoxantrone (now referred to as CM)
AML	Acute myeloid leukemia
ANC	Absolute neutrophil count
ANOVA	Analysis of variance
AP	Accelerated phase
APL	Acute promyelocytic leukemia
Ara-c	Cytarabine
AST	Aspartate aminotransferase
AUC	Area under the curve
BID	Every 12 hours
BM	Bone marrow
BUN	Blood urea nitrogen
CBC	Complete blood count
CHR	Complete hematologic remission
Cl	Clearance
CLL	Chronic lymphocytic leukemia
CM	**C**ytarabine/**M**itoxantrone (previously referred to as AM)
CNS	Central nervous system
COV	Close out visit
CR	Complete remission/response
CRA	Clinical research associate
CRi	Complete remission with residual neutropenia (ANC <1000 µL) or lack of recovery of any other hematopoietic cell
CRF	Case report form
CRp	Complete remission with incomplete platelet recovery
CTCAE	Common Terminology Criteria for Adverse Events
DCF	Data clarification form
DLT	Dose-limiting toxicity
DNA	Deoxyribonucleic acid
DSMB	Data Safety Monitoring Board
ECHO	Echocardiogram
ECOG	Eastern Cooperative Oncology Group
EFS	Event-free survival
EMD	Extramedullary disease
FDA	Food and Drug Administration
FLAM	Alvocidib (**Fl**avopiridol)/**A**ra-c/**M**itoxantrone (now referred to as ACM)
GCP	Good Clinical Practice
GCSF	Granulocyte colony stimulating factor
GM-CSR	Granulocyte-macrophage colony stimulating factor
GVHD	Graft versus host disease
HCG	Human chorionic gonadotropin
HCT	Hematocrit

Hgb	Hemoglobin
HI	Hematologic improvement
IC_{50}	Inhibitory concentration in 50% of animals
IEC	Independent Ethics Committee
IND	Investigational New Drug Application
IP	Intraperitoneal
IRB	Institutional Review Board
IT	Intrathecal (chemotherapy)
ITT	Intent to treat (population)
LD_{50}	Lethal dose in 50% of animals
LDH	Lactate dehydrogenase
LR	Logistic regression
LVEF	Left ventricular ejection fraction
MedDRA	Medical Dictionary for Regulatory Activities
MDS	Myelodysplastic syndrome
MRD	Minimal residual disease
MTD	Maximum tolerated dose
MUGA	Multigated acquisition (scan)
NCCN	National Comprehensive Cancer Network
NCI	National Cancer Institute
OS	Overall survival
PR	Partial remission/response
PS	Performance status
RBC	Red blood cell
RFS	Relapse-free survival
RNA	Ribonucleic acid
RT-PCR	Reverse transcription-polymerase chain reaction
SAE	Serious adverse event
SAS	Statistical Analysis System (software)
SGOT (AST)	Serum glutamic-oxaloacetic transaminase
SGPT (ALT)	Serum glutamic-pyruvic transaminase
SIV	Study initiation visit
SOP	Standard operating procedure(s)
$T_{1/2}$	Half-life
Tmax	Time to maximum concentration
TRM	Treatment-related mortality
TST	Timed sequential therapy
ULN	Upper limit of normal
Vd	Volume of distribution
Vss	Volume at steady state
WBC	White blood cell
WHO	World Health Organization

STUDY SUMMARY

Title of Study:	A Phase 2, Randomized, Biomarker-driven, Clinical Study in Patients with Relapsed or Refractory Acute Myeloid Leukemia (AML) with MCL-1 Dependence ≥***30***%
Study Indication:	• Relapsed or primary refractory AML with MCL-1 dependence of ≥***30***% demonstrated by mitochondrial profiling
Objectives:	**Stage 1: Primary Objective** • To determine the CR rate in patients with relapsed or refractory AML with MCL-1 dependence of ≥***30***% **Stage 2: Primary Objective** • To compare CR rates between patients with relapsed or refractory AML with MCL-1 dependence of ≥***30***% receiving 1 cycle of ACM treatment and those receiving 1 cycle of CM. **Exploratory Objective (Stage 2):** • To determine if treatment with ACM can induce CR in patients with relapsed or refractory AML with MCL-1 dependence of ≥***30***% who failed to achieve CR following 1 cycle of CM.
Clinical Phase:	2
Study Design:	*"FLAM" (alvocidib, cytarabine, mitoxantrone hydrochloride) will now be referred to as "ACM".* *"AM" (cytarabine, mitoxantrone hydrochloride) will now be referred to as "CM".* This is an open-label, randomized, multicenter two-stage study of ACM (Stage 1) and ACM versus CM (Stage 2) in patients with acute myeloid leukemia (AML) with demonstrated MCL-1 dependence of ≥***30***% by mitochondrial profiling in bone marrow who are either in first relapse (within 24 months of CR) or have failed induction therapy***** (no CR or CRi after treatment with an intensive regimen [eg, anthracycline/cytarabine ± etoposide, gemtuzumab ozogamicin, or cladribine]). *Induction therapy may involve 1 or 2 cycles of the same regimen. Efficacy assessment of induction therapy must be >21 days from the start of the previous induction cycle. Stage 1 of the study will be single arm, conducted similarly to the first stage of a Simon 2-stage design. All patients with relapsed or refractory AML enrolled in this stage will receive ACM. Once ≥13 patients enrolled in Stage 1 exhibit a CR, CRi, or CRp, enrollment into Stage 1 will close and additional patients will be enrolled in Stage 2 of the study. Enrollment into Stage 1 will be limited to no more than 23 evaluable patients, however enrollment into Stage 2 may be initiated at any

	point after confirming CR, CRi or CRp responses in 13 Stage-1 patients. The sample size of 23 eligible patients for Stage 1 was selected because it allows estimation of the remission rate by means of a 90% confidence interval with maximum width of ±17%. Although this study does not follow a Simon 2-stage design, 23 patients is consistent with the sample size for Stage 1 of the Simon 2-stage minimax design with 80% power for testing the null hypothesis that the remission rate is 50% against a one-sided alternative at the 5% level of significance when the actual remission rate is 70%. In the Simon 2-stage design described, the study would continue to Stage 2 only if 13 patients achieve remission out of 23 eligible patients. Therefore, this was selected as the requirement to proceed to Stage 2 of this study. However, the study may proceed to Stage 2 as soon as 13 patients achieve remission. In Stage 2, patients will be randomized to receive either ACM or CM. The planned sample size of 56 patients (28 per treatment arm) in Stage 2 of this study provides 90% power to reject the null hypothesis of equal CR rates across treatment arms under the following conditions: • CR rates of 70% and 30% for patients receiving ACM and CM, respectively • One-sided 2.5% significance level • Randomization based on a 1:1 patient allocation ratio (ACM to CM) • No interim analysis Randomization to treatment arm will be stratified by: • Response to first-line therapy: ○ Refractory: persistent disease or complete remission [CR] duration <90 days ○ Early relapse: CR duration 90 days to 1 year ○ Late relapse: CR duration >1 year but <24 months Response assessments in Stage 1 are defined by the International Working Group Criteria and 2010 European LeukemiaNet (ELN). The 2017 ELN criteria will be used to determine patient responses during Stage 2. Response assessments will include: • CR rate = Percentage of patients achieving CR • Combined CR rate (CR+CRp†+CRi) • Combined Response Rate (CR+CRp†+CRi+PR) • Overall Survival (OS) • Event-free Survival (EFS) • Rate of Stem Cell Transplantation

	†Note that CRp has been retained in definitions for Combined CR Rate and Combined Remission Rate because Stage-1 patients were assessed using the IWG criteria. CRp will not be used for assessing response in Stage-2 patients and, therefore, will not be included in the definitions of Combined CR Rate and Combined Remission Rate in Stage 2. Safety assessments will include: • Mortality from any cause at 30 and 60 days • Adverse events graded according to National Cancer Institute's Common Terminology Criteria for Adverse Events (NCI CTCAE), version 4.03 in Stage 1 and version 5.0 in Stage 2 o The multivariate analysis and risk score prediction model by Montesinos and colleagues [22] will be used to assess the potential for development of TLS (*Appendix F*) Treatment assessments will include: • Bone marrow biopsies and/or aspirates will be performed before treatment and at hematologic recovery (ie, absolute neutrophil count (ANC) >1000 µL and platelet count >100,000 µL) or Day 45, whichever occurs first. • Complete blood counts and chemistries assessed daily during hospitalization for chemotherapy administration and weekly thereafter • Overall survival (OS) monitored monthly during year 1, every 2 months during year 2 • Intensive monitoring of renal function, electrolytes, potassium, and uric acid levels Pharmacodynamic assessments will include: • Determination of MCL-1 dependence at baseline using bone marrow Patients who achieve CR, CRi, CRp, or PR after the first cycle (completion of all doses) may receive up to 3 additional optional cycles of treatment. After completing the first cycle of treatment, continued use of mitoxantrone is optional. Mitoxantrone must be omitted from subsequent cycles if the patient's lifetime daunorubicin equivalent exceeds 460 mg/m^2 (see *Appendix G* for conversion table) or the left ventricular ejection fraction (LVEF) drops below 45%. Patients not demonstrating evidence of CR, CRi, CRp, or PR after the first cycle of treatment will be considered for removal from the study, although with permission of the Medical Monitor, induction treatment

	may continue if clinically indicated and provided there is no evidence of toxicity ≥NCI CTCAE grade 4. *Crossover:* Patients randomized to CM with progressive disease or no response after 1 cycle of CM may cross over to receive ACM. In addition, patients randomized to CM with a best response of PR after 2 cycles of CM may also cross over to receive ACM. Patients who cross over to ACM may receive up to a combined total (CM + ACM) of 4 cycles. Results from Stage 1 (ie, the Primary Relapsed/Refractory AML Study Arm) will be analyzed using descriptive statistics and confidence intervals. In Stage 2, complete remission (CR) rates will be compared across treatment groups using the Cochran-Mantel-Haenszel general association test stratified by response to most recent induction therapy. The same test will be applied to assess the statistical significance of the secondary response rate endpoints. Control of the overall one-sided 2.5% type-1 error rate for multiple comparisons will be achieved using the hierarchical closed test procedure. Primary statistical comparisons of efficacy results will use only data from Stage 2; however, data from Stage 1 patients will be combined with data from patients receiving ACM in Stage 2 for secondary efficacy analyses. Treatment-group distributions of time-to-event endpoints will be compared using the log-rank test stratified by response to most recent induction therapy. Patients who are lost to follow-up or have not had the event of interest at the time of analysis will have their event time censored (see Statistical Methods).
Patient Population:	Patients will be prescreened to determine percent MCL-1 dependence. • Patients with AML with demonstrated MCL-1 dependence of ≥***30***% by mitochondrial profiling in bone marrow who are either in first relapse (within 24 months of CR) or have failed induction therapy***** (no CR or CRi after treatment with an intensive regimen (eg, anthracycline/cytarabine ± etoposide, gemtuzumab ozogamicin, or cladribine) o *Induction therapy may involve 1 or 2 cycles of the same regimen. Efficacy assessment of induction therapy must be >21 days from the start of the previous induction cycle.
Inclusion Criteria:	To be eligible for participation in the study, patients must meet all of the following inclusion criteria: <u>During Prescreening:</u> 1. Be between the ages of ≥18 and ≤65 years

	2. Have an established, pathologically confirmed diagnoses of AML by World Health Organization (WHO) criteria excluding acute promyelocytic leukemia (APL-M3) with a bone marrow of >5% blasts based on histology or flow cytometry 3. Be in first relapse (within 24 months of CR) or have failed induction therapy* (no CR or CRi after treatment with an intensive regimen [eg, anthracycline/cytarabine ± etoposide, gemtuzumab ozogamicin, or cladribine]) *Induction therapy may involve 1 or 2 cycles of the same regimen. Efficacy assessment of induction therapy must be >21 days from the start of the previous induction cycle. 4. Demonstrate MCL-1 dependence of ≥***30***% by mitochondrial profiling in bone marrow During Screening: 5. Have an Eastern Cooperative Oncology Group (ECOG) performance status (PS) ≤2 6. Have a serum creatinine level ≤1.8 mg/dL 7. Have an alanine aminotransferase (ALT)/aspartate aminotransferase (AST) level ≤5 times upper limit of normal (ULN) 8. Have a total bilirubin level ≤2.0 mg/dL (unless secondary to Gilbert syndrome, hemolysis, or leukemia) 9. Have a left ventricular ejection fraction (LVEF) >45% by echocardiogram (ECHO) or multigated acquisition (MUGA) scan 10. Be nonfertile or agree to use an adequate method of contraception. Sexually active patients and their partners must use an effective method of contraception associated with a low failure rate during and for at least 6 months after completion of study therapy (see *Section 4.7.3*). 11. Be able to comply with the requirements of the entire study. 12. Provide written informed consent prior to any study related procedure. (In the event that the patient is re-screened for study participation or a protocol amendment alters the care of an ongoing patient, a new informed consent form must be signed.)
Exclusion Criteria:	Patients meeting any one of these exclusion criteria will be prohibited from participating in this study. 1. Received more than 2 cycles of induction therapy for AML. Investigational agents as part of front-line therapy for AML may by acceptable following discussion with the Medical Monitor. Hydroxyurea is permitted (see #5 below). 2. Received any previous treatment with alvocidib or any other CDK inhibitor 3. Received a hematopoietic stem cell transplant within the previous 2 months

	4. Have clinically significant graft versus host disease (GVHD), or GVHD requiring initiation or escalation of treatment within the last 21 days 5. Require concomitant chemotherapy, radiation therapy, or immunotherapy. Hydroxyurea is allowed up to the evening before starting (but not within 12 hours) of starting treatment on either arm. 6. Received >360 mg/m^2 equivalents of daunorubicin (see *Appendix G* for conversion table) 7. Have a peripheral blast count of >30,000/mm^3 (may use hydroxyurea as in #5 above) *8.* Received antileukemic therapy within the last 3 weeks *(*with the exception of hydroxyurea or if the patient has definite refractory disease). Refractory patients who received therapy within the last 3 weeks may be eligible with prior approval of the Medical Monitor. 9. Diagnosed with acute promyelocytic leukemia (APL, M3) 10. Have active central nervous system (CNS) leukemia 11. Have evidence of uncontrolled disseminated intravascular coagulation 12. Have an active, uncontrolled infection 13. Have other life-threatening illness 14. Have other active malignancies or diagnosed with other malignancies within the last 6 months, except nonmelanoma skin cancer or cervical intraepithelial neoplasia 15. Have mental deficits and/or psychiatric history that may compromise the ability to give written informed consent or to comply with the study protocol. 16. Are pregnant and/or nursing 17. Have received any live vaccine within 14 days prior to first study drug administration
Study Treatment:	Stage 1 (the Primary Relapsed/Refractory AML Study Arm): • All patients enrolled in Stage 1 will receive ACM. **A**: alvocidib (formerly flavopiridol), 30 mg/m^2 as a 30-minute (±10 minutes) intravenous (IV) bolus followed by 60 mg/m^2 over 4 hours (±15 minutes) as an IV infusion administered daily on Days 1-3 **C**: cytarabine (Ara-c), 2 gm/m^2 by continuous IV infusion over 72 hours (±2.5 hours) on Days 6-8 (ie, 667 mg/m^2 daily on Days 6, 7, and 8 for total of 2 gm/m^2) **M**: mitoxantrone (mitoxantrone hydrochloride), 40 mg/m^2 by IV infusion over 1-2 hours (±10 minutes) starting 12 hours after completing cytarabine

	Stage 2 (ACM vs AM): • Patients with AML with demonstrated MCL-1 dependence of ≥40% by mitochondrial profiling in bone marrow, who are enrolled in Stage 2, will be randomized 1:1 to receive either ACM (dosing as described in Stage 1) or CM. **C**: cytarabine (Ara-c), 2 gm/m^2 by continuous IV infusion over 72 hours (±2.5 hours) on Days 1-3 (ie, 667 mg/m^2 daily on Days 1, 2, and 3 for total of 2 gm/m^2) **M**: mitoxantrone (mitoxantrone hydrochloride), 40 mg/m^2 by IV infusion over 1-2 hours (±10 minutes) starting 12 hours after completing cytarabine • *Crossover:* Patients randomized to CM who exhibit progressive disease or no response after a single cycle of CM may cross over to receive ACM. In addition, patients randomized to CM with a best response of PR after two cycles of CM may also cross over to receive ACM. Patients who cross over to ACM may receive up to a combined total (CM + ACM) of 4 cycles. Supportive care will be provided including: • Tumor Lysis Prevention and Treatment o Mandatory IV hydration with 0.45% NaCl (or similar hydration fluid per institutional standard) sterile solution at 100 cc/hour for at least 10 hours prior to initiation of first dose of chemotherapy during Cycle 1 (optional for subsequent cycles) in both treatment arms. If, by Day 4, there is no evidence of tumor lysis syndrome, the hydration rate can be reduced to a maintenance level. • Diligent monitoring of urine output **frequently** to ensure that it equals fluid input. If input is greater than output by 10%, administration of diuretics is encouraged. Replacement of excessive fluid losses, including from diarrhea, should be done unless otherwise clinically indicated. • Mandatory allopurinol orally each day of dosing during Cycle 1 (optional in subsequent cycles) to be started at same time as initiation of IV hydration in all treatment arms. • Mandatory oral phosphate binder to be started at the same time as initiation of IV hydration during Cycle 1 (optional in subsequent cycles) in all treatment arms, unless contraindicated. • Evaluation of laboratory indicators of tumor lysis syndrome (TLS) during Cycle 1: o Tumor lysis laboratory evaluations (tumor lysis labs) include electrolytes (sodium, potassium, chloride, and carbon dioxide) as well as creatinine, calcium, lactate dehydrogenase (LDH), uric acid, and phosphorus levels

	○ Monitor tumor lysis labs at the start of treatment (alvocidib for ACM, cytarabine for CM) and throughout the treatment cycle according to the schedule outlined in *Section 4.6.1.1.* [Note: there are different blood collection schedules for monitoring of TLS labs for patients at high risk of TLS and for those NOT at high risk for TLS.] ○ Monitor fibrinogen levels at baseline and then as clinically indicated • Infection Prevention ○ Prophylactic antibiotics including levofloxacin (or equivalent) 500 mg orally once daily and valacyclovir (or equivalent) 500 mg orally BID each day should be administered to patients in all treatment arms at the start of chemotherapy. Alternative prophylactic antibiotic and antiviral therapy is left to the discretion of the treating physician and institutional standards. ▪ Testing for *Clostridioides difficile* (*C. difficile*) should start with first episode of diarrhea. GI antibiotics should then be initiated based on the results of the *C. difficile* testing. ○ To prevent cytarabine-related conjunctivitis, corticosteroid eye drops per institutional formulary (eg, prednisolone 0.1%) must begin 1 day prior to institution of cytarabine infusion (ie, Day 6 for ACM; Day 1 for CM) and continue for at least 7 days ○ Antifungal prophylaxis to be administered according to each institution's standard of care ○ Routine growth factor support is not allowed ≤Day 35 of therapy. Growth factor support can be given at the discretion of the Investigator >Day 35 of therapy in the presence of life-threatening infection with ongoing neutropenia. ○ Donor lymphocyte infusions are not allowed unless discussed and approved by the medical monitor. Suggested doses of these supportive care therapies are provided in the protocol; however, adjustment of the dosages based on the patient's clinical condition or each institution's standard of care is permitted.
Efficacy Evaluations:	**Primary Endpoint** The primary endpoint is the rate of Complete Remission (CR) after Cycle 1 as defined in Stage 1 by the International Working Group Criteria and 2010 ELN criteria and in Stage 2 by the 2017 ELN criteria (*Appendix E*). **Secondary Endpoints** • Combined CR Rate = Percentage of patients achieving: ○ CR = Bone marrow blasts <5%; absence of blasts with Auer rods; absence of extramedullary disease; hematologic

	recovery (absolute neutrophil count [ANC] >1000 µL and platelet count >100,000 µL); independence of red cell transfusions (In Stage 2, per 2017 ELN, independence of red cell transfusions is not a requirement for CR); plus o CRi = Meets all CR criteria except for residual neutropenia (ANC <1000 µL) or lack of recovery of any other hematopoietic cell; plus o CRp = Meets all CR criteria except for residual thrombocytopenia (platelet count <100,000 µL) † • Combined Response Rate = Percentage of patients achieving: o CR o CRi o CRp † o PR = Meets all hematologic values required for CR but with a decrease of at least 50% in the percentage of blasts to ≥5% to ≤25% in bone marrow • Overall Survival (OS) = Time from randomization (Day 1) until death from any cause • Event-free Survival (EFS) = Time from randomization (Day 1) until (a) treatment failure, (b) relapse after CR, or (c) death from any cause, whichever occurs first, censored at 2 years • Rate of Stem Cell Transplantation = Percentage of patients proceeding directly to stem cell transplantation †Note that CRp has been retained in definitions for Combined CR Rate and Combined Remission Rate because Stage-1 patients were assessed using the IWG criteria. CRp will <u>not</u> be used for assessing response in Stage-2 patients and, therefore, will not be included in the definitions of Combined CR Rate and Combined Remission Rate in Stage 2. The CR rate in patients failing 1-2 cycles of CM and crossing over to receive ACM will also be determined. The Complete Remission (CR) rate, defined as the percentage of patients achieving CR, will be determined. Secondary endpoints described for Stage 1 will also be assessed. Complete details of the planned analysis will be documented in a full Statistical Analysis Plan, which will be finalized before locking the study database.
Safety Evaluations:	Safety and tolerability of the ACM and CM regimens will be assessed by analyzing the incidence rates of treatment-emergent adverse events summarized within treatment groups at the MedDRA preferred term and primary system organ class levels. Similar summaries will be made for subsets of AEs such as (1) those judged by the Investigator to be related to study treatment, and (2) serious adverse events (SAEs). The multivariate analysis and risk score prediction

	model by Montesinos and colleagues [22] will be used to assess the potential for development of TLS (*Appendix F*). Other routine safety assessments (eg, clinical laboratory parameters and vital signs) will be summarized by shift tables and treatment group using mean, standard deviation, median, minimum and maximum changes from baseline values. Mortality (all causes) at 30 and 60 days will also be calculated. A Data Safety Monitoring Board (DSMB) will monitor key outcomes from the study.
Study Duration:	The study is expected to take 20-24 months to enroll up to 137 patients: 25 patients in Stage 1; 56 patients in the 4 Exploratory Arms (closed after Amendment 8); and 56 patients (28 patients in each of two treatment arms in Stage 2.

1. INTRODUCTION

1.1 Acute Myeloid Leukemia (AML)

The clinical objective of induction chemotherapy is to obtain 'first CR' (complete remission with recovery of blood counts) in as many newly diagnosed AML patients as possible. Attaining initial CR enables application of further therapies of curative intent (consolidation and transplant). Once CR is obtained, survival also varies as a function of age, cytogenetic risk status, and possibly, by minimal residual disease (MRD). In patients with unfavorable risk AML, <60% achieve CR, many patients relapse quickly, and survival among responders is woefully low with 5% to 10% experiencing chemotherapy-related deaths due to severe toxicity. These data amplify the decades' long tragedy and debilitating nature of available treatments for newly diagnosed AML patients, particularly for older patients >60 years, those with adverse and complex cytogenetics, and patients with secondary AML.

1.2 Relapsed/refractory AML

As described above, a significant number of patients receiving initial induction chemotherapy for AML either do not achieve remission or relapse after induction of remission.

Beginning in the late 1990s, intermediate- and high-dose cytarabine has produced approximately 45% CRs and 5 months median survival in relapsed/refractory AML patients. Several combinations with experimental agents in the 2000s did not achieve any added benefit, in fact, the CR rates for these combinations were lower than 40%, and this observation continued in 2010 with the publication of E4999, where the CR rates for three combination regimens did not exceed 12%. In 2012, MD Anderson published results with combination of clofarabine and intermediate-dose cytarabine which produced a 47% CR and over 6 months' survival. In 2012, The University of Chicago published results of age-specific doses of intermediate and high-dose cytarabine in combination with mitoxantrone. Among the 19 relapsed/refractory patients treated, the CR + CRi rate was 58%.

At the ASH 2014 meeting, the randomized Phase 3 VALOR trial was presented, comparing intermediate-dose cytarabine with or without vosaroxin in 711 relapsed/ refractory AML patients. The vosaroxin combination produced 30% CR, compared to 16% CR with intermediate-dose cytarabine alone. OS was higher for the vosaroxin combination (7.5 months versus 6 months, although not statistically (p=0.06).

1.3 Role of MCL-1 in AML

The ability to resist cell death is an important hallmark of all cancer cells, allowing them to divide uncontrollably [1, 2]. The BCL-2 family of polypeptides has been widely studied as regulators of cell death [3]. Because members of this protein family can exhibit pro- or anti-apoptotic functions, they are highly involved in the development of cancer and in mechanisms of resistance to many therapeutic agents. The myeloid leukemia cell-1 (MCL-1) gene is the predominant BCL2 family member expressed in primary AML samples. Tumor resistance to targeted therapies limits effectiveness of current clinical regimens. Overexpression of MCL-1 has been shown to convey resistance to apoptosis induced by a number of different treatments, including etoposide, in vitro [4, 5]. Other studies have indicated that MCL-1 expression can be induced rapidly in response to a number of DNA-damaging agents [6, 7].

Alvocidib (flavopiridol), a potent cyclin-dependent kinase (CDK) inhibitor, downregulates the expression of MCL-1 through inhibition of CDK 9, which, in turn, inhibits tumor growth. Studies have shown that alvocidib suppresses MCL-1 expression [8, 9] and acts synergistically with other chemotherapeutic agents like daunorubicin. Through use of mitochondrial (BH3) profiling, the mechanism by which apoptosis is suppressed in a population of cells can be determined. Mitochondrial sensitivity to NOXA BH3 peptides suggests dependence on MCL-1 to mediate resistance to apoptosis. Early studies have shown that MCL-1 dependence can predict the clinical activity of alvocidib in AML patient samples and suggests an important role for MCL-1 activity in predicting alvocidib activity.

In light of these findings, Tolero is conducting this Phase 2 study to determine if MCL-1 dependency determined by mitochondrial profiling of bone marrow samples will increase the Complete Remission (CR) rate after FLAM (ACM) treatment.

1.4 Rationale

Acute myeloid leukemia continues to be one of the highest unmet medical needs, due to very short survival from time of diagnosis (median <1 to 2 years). Short survival in AML patients is directly correlated with the presence of unfavorable cytogenetics and multiple adverse clinical features (age >60, prior MDS, treatment-related or secondary AML, FLT3-ITD positivity, and monocytic phenotype). Attainment of initial complete remission still remains unachievable in approximately 40-60% of AML patients, and progression, rapid relapse and short survival are too frequent consequences from currently available, but inadequate, antileukemic therapy.

Tolero's position remains that the existing AML therapies developed decades ago, which remain the standard of care, do not adequately attain sufficiently high rates of first CR, long-term remission nor survival for the majority of AML patients. In treating relapsed/refractory AML, current therapies produce

modest complete remission rates, and almost all of these patients succumb to disease-related mortality. As noted in the 2014 National Comprehensive Cancer Network (NCCN) Guidelines for patients with AML, the best option for patients with relapsed or refractory AML is a clinical trial.

Our experience to date with alvocidib, as part of the FLAM regimen, clearly shows that this novel treatment can produce exceptionally high complete remission rates:

- 62 - 80% in newly diagnosed unfavorable risk AML patients based on three Phase 2 studies
- 29 - 92% in patients with relapsed AML
- 28 - 43% in combined populations of relapsed/refractory AML

In relapsed AML patients, where no standard of care exists, FLAM has produced CR rates as high as 75 - 92%. Among relapsed/refractory AML patients treated with the alvocidib/FLAM hybrid dosing regimen, a 50% CR rate was observed among 24 patients treated at dose level 5 (30 and 40 mg/m^2 hybrid dosing in FLAM) [10]. This clinical activity has allowed many patients the opportunity to undergo stem cell transplantation, who otherwise may not have had this option.

2. DRUG INFORMATION – ALVOCIDIB

A comprehensive review of Alvocidib is contained in the Investigator's Brochure provided by the Sponsor. This document should be reviewed prior to initiating the study.

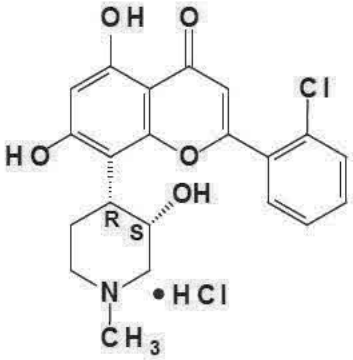
2.1 Background

Alvocidib (formerly flavopiridol) was discovered and synthesized from an alkaloid isolated from the stems and leaves of *Dysoxylum binectariferum* (India). Dr. Edward Sausville and colleagues at the National Cancer Institute (NCI) first determined alvocidib cell cycle arrest/growth inhibition properties in 1992.

2.2 Chemistry

Generic Name:	Alvocidib hydrochloride
Chemical Name:	2-(2-Chlorophenyl)-5,7-dihydroxy-8-[(3S, 4R)-3-hydroxy-1-methyl-4-piperidinyl]-4H-chromen-4-one, hydrochloride
Other Names:	Flavopiridol
CAS Registry Number:	131740-09-5
Formula:	$C_{21}H_{20}ClNO_5$, HCl
Molecular Weight:	438.31 (salt), 401.85 (active moiety)

Structure:



2.3 Drug Description

Alvocidib hydrochloride is supplied as a sterile, intravenous, nonpyrogenic, yellow-colored aqueous solution. Each vial contains 50 mg of alvocidib base (10 mg/mL). Alvocidib is to be diluted with either 0.9% sodium chloride injection or 5% dextrose injection prior to infusion, providing solutions of 0.09 to 1.0 mg/mL alvocidib.

2.4 Mechanism of Action

Alvocidib is a potent cyclin-dependent kinase (CDK) inhibitor with selectivity for CDKs 9, 1, 2, 4 and 7. The greatest inhibition (IC_{50} of 6 nM) was observed with CDK 9. Alvocidib-induced apoptosis results at least in part from inhibition of multiple serine-threonine CDKs leading to changes in gene expression of critical survival and proliferative genes including BCL-2, myeloid cell leukemia-1 (MCL-1) and c-myc. Whereas inhibition of CDK 2 and CDK 4 contributes to cell cycle arrest in G1 and G2, alvocidib-triggered inactivation of the CDK 9/cyclin T complex (also known as PTEF-b) inhibits the activating phosphorylation of RNA polymerase 2 and diminishes mRNA synthesis. Consequently, alvocidib-treated cells are unable to synthesize transcripts encoding polypeptides, such as cyclin D1 and c-myc, which are expressed in a cell cycle-dependent manner.

Inhibition of CDK 9, which is involved in the regulation of transcription by RNA polymerase 2, is postulated to be a key event in the inhibition of transcription observed following alvocidib treatment. Effects on CDK 9 may be particularly relevant to inducing apoptosis in malignant hematopoietic cells.

Because alvocidib induces cell cycle arrest, it antagonizes the effects of S-phase–dependent agents, such as cytarabine and topotecan, when administered concomitantly. In contrast, when alvocidib is administered first and then withdrawn in vitro, the surviving cells re-enter the cell cycle and are sensitized to S-phase poisons.

These observations, coupled with the ability of alvocidib to kill non-cycling cells, suggested that alvocidib might be particularly effective when administered first and then followed several days later by cytarabine. Therapeutically achievable alvocidib concentrations induced apoptotic cell death in bone marrow leukemic blasts in vitro and that alvocidib-treated blast cultures exhibited increased sensitivity to the subsequent pro-apoptotic effects of cytarabine relative to either agent alone.

2.5 Preclinical Studies

2.5.1 In vitro/in vivo studies

[redacted]

[redacted]

2.5.2 Safety Pharmacology

[redacted]

2.5.3 Nonclinical Absorption, Distribution, Metabolism and Excretion

[redacted]

2.5.4 Animal Toxicology

[redacted]

2.5.5 Genotoxicity

[redacted]

2.5.6 Reproductive and Developmental Toxicity

[redacted]

2.5.7 Other Toxicity Studies

[redacted]

[redacted]

2.6 Clinical Studies

Alvocidib has now been evaluated in solid tumors and hematologic malignancies. Eight Phase 1 and 2 clinical trials have been completed in patients with intermediate and poor-risk AML, including more than 400 patients with both relapsed/refractory and newly diagnosed AML. In these trials, alvocidib has been evaluated as a single agent as well as in combination with cytarabine and mitoxantrone.

2.6.1 Phase 1 and 2 Clinical Studies of Bolus and Hybrid FLAM Regimens in Patients with AML

Initially, Phase 1 clinical trials in AML patients incorporated alvocidib into the "Timed Sequential Therapy" (TST) AML induction therapy approach from the 1990s, which had utilized cytarabine and later added mitoxantrone (AM) [11]. Investigators at the University of Maryland, and then at Johns Hopkins, added alvocidib to AM for the dual purpose of initial cytoreduction and enhancing the cell cycle progression of the remaining leukemic cell cohort, followed by the cycle-dependent agents cytarabine and mitoxantrone (FLAM regimen). Two alvocidib dosing schedules have been evaluated: by 1-hour *bolus* infusion, and by a *hybrid* dosing schedule consisting of a 30-minute short IV bolus dose followed by a 4-hour IV infusion. A listing of all eight (8) clinical studies of FLAM in relapsed/refractory patients and newly diagnosed patients is provided in Table 1.

Table 1: Overview of Alvocidib Phase 1 & 2 Clinical Studies in AML (In Chronologic Order)

Study (Reference)	N	Treatment Regimen	Patient Population
Study 1: JHOC J0254/ NCI-3170 Phase 1 FLAM [12]	Total: 34 AML: 26	Alvocidib Bolus 1 hr IV: 40, 50, 60 mg/m^2/d on Days 1-3 Ara-c: 2 gm/m^2/72h by CIV given Days 6-8 Mitoxantrone: 40 mg/m^2 given by 60-120 min IV on day 9	Adults median age 54, primary refractory, multi-refractory or relapsed AML (26) ALL (7) CML (1)
Study 2: JHOC J0254/ NCI-3170 Phase 2 FLAM [13]	62 AML	Alvocidib Bolus 1 hr IV: 50 mg/m^2/d on Days 1-3 Ara-c: 2 gm/m^2/72h by CIV given Days 6-8 Mitoxantrone: 40 mg/m^2 given by 60-120 min IV on day 9	Adults median age 58, primary refractory (13), multirefractory (10), relapsed (24), newly diagnosed secondary AML (15)
Study 3: OSU-0479/ NCI-6947 Phase 1 Alvocidib Monotherapy [14]	Total: 24 AML: 19	Alvocidib monotherapy dose-escalation, Hybrid regimen: 20 mg/m^2 & 30 mg/m^2 30 mg/m^2 & 35 mg/m^2 30 mg/m^2 & 50 mg/m^2 40 mg/m^2 & 60 mg/m^2 50 mg/m^2 & 75 mg/m^2 30 min bolus followed by 4-hr infusion/day on Days 1,2,3	Adults median age 62, relapsed or refractory non-M3 AML (19), ALL (5)
Study 4: JHOC J0669/ NCI-7845 Phase 2 FLAM [15]	45 AML	Alvocidib Bolus 1hr IV: 50 mg/m^2/d Days 1-3 Ara-c: 2 gm/m^2/72h by CIV given Days 6-8 Mitoxantrone: 40 mg/m^2 given by 60-120 min IV on day 9	Adults median age 61, newly diagnosed, pathologically confirmed, previously untreated intermediate/poor risk AML
Study 5: JHOC J06133/ NCI-7889 Phase 1 FLAM [10]	Total: 55 AML: 49	Alvocidib dose-escalation in Hybrid regimen 20 mg/m^2 & 30 mg/m^2 25 mg/m^2 & 35 mg/m^2 30 mg/m^2 & 40 mg/m^2 30 mg/m^2 & 50 mg/m^2 30 mg/m^2 & 60 mg/m^2 30 mg/m^2 & 70 mg/m^2 given as: 30-min bolus followed by 4-hr infusion/d on Days 1,2,3 Ara-c: 2 gm/m^2/72h by CIV given Days 6-8 Mitoxantrone: 40 mg/m^2 given by 60-120 min IV on day 9	Adults median age 54, pathologically confirmed relapsed and refractory AML (49), ALL (3), ABL (3)

Table 1: Overview of Alvocidib Phase 1 & 2 Clinical Studies in AML (In Chronologic Order) (cont)

Study (Reference)	N	Treatment Regimen	Patient Population
Study 6: ECOG 1906 Phase 2 Randomized Trial of Carboplatin and Topotecan; Alvocidib, Mitoxantrone and Cytosine Arabinoside; and Sirolimus, Mitoxantrone, Etoposide and Cytosine Arabinoside for the Treatment of Adults With Primary Refractory or Initial Relapse of AML [16] *Ongoing follow-up*	AML Total: 111 Arm B FLAM: 36	Arm A: *CT* carboplatin and topotecan IV continuously over 24 hours on days 1-5 Arm B: *Hybrid FLAM* Alvocidib: 30 mg/m^2 by 30-min bolus followed by & 60 mg/m^2 by 4-hr CIV/day on Days 1,2,3 Ara-c: 2 gm/m^2/72h by CIV given Days 6-8 Mitoxantrone: 40 mg/m^2 given by 60-120 min IV on day 9 Arm C: *Sirolimus-MEC* sirolimus PO QD on days 2-9, mitoxantrone hydrochloride IV over 15 minutes QD, etoposide IV over 1 hour QD, and Ara-c IV over 3 hours QD on days 4-8 or 5-9	Adults 18-70 years, relapsed or refractory AML (36 on FLAM arm); median age 58
Study 7: JHOC J0856/ NCI-8237 Phase 2 FLAM [17]	AML 78	Arm A: *Bolus FLAM* Alvocidib Bolus 1 hr IV: 50 mg/m^2/d on Days 1-3 Ara-c: 2 gm/m^2/72h by CIV given Days 6-8 Mitoxantrone: 40 mg/m^2 given by 60-120 min IV on day 9 Arm B: *Hybrid FLAM* Alvocidib: 30 mg/m^2 by 30-min bolus followed by 40 mg/m^2 by 4-hr CIV/day on Days 1,2,3 Ara-c: 2 gm/m^2/72h by CIV given Days 6-8 Mitoxantrone: 40 mg/m^2 given by 60-120 min IV on day 9	Adults median age 61, newly diagnosed, pathophysiologically confirmed, previously untreated intermediate/poor risk AML
Study 8: JHOC J1101/ NCI-8972 Randomized Phase 2 FLAM vs 7&3 [18] *Ongoing follow-up*	AML Total: 165 FLAM: 109 7&3: 56	Arm A: *Bolus FLAM* Alvocidib Bolus 1-hr IV: 50 mg/m^2/d on Days 1-3 Ara-c: 2 gm/m^2/72h by CIV given Days 6-8 Mitoxantrone: 40 mg/m^2 given by 60-120 min IV on day 9 Arm B: 7&3 Ara-c: 100 mg/m^2/day IV infusion Days 1-7 Daunorubicin 90 mg/m^2 /day IV over 30-60 minutes Days 1, 2, 3	Adults median age 60 (FLAM), newly diagnosed, pathologically confirmed, previously untreated intermediate/poor risk AML (including Secondary AML)

2.6.2 Data Summaries from Phase 1 and 2 Clinical Studies in Patients with Relapsed/Refractory AML

Table 2, Table 3, and Table 4 provide details regarding the demographics, efficacy, and safety data, respectively, summarized from the five trials of FLAM in relapsed/refractory AML patients (ie, Studies 1, 2, 3, 5, and 6).

Table 2: Alvocidib/FLAM in AML (Demographics and Dosing in Relapsed/Refractory Patients)

List of Studies	Demographics and Dosing				
	Alvocidib Dosing	*AML* pts per DL	Stage of Disease	Secondary AML	Adverse Cytogenetics
STUDY 1: JHU-0254 / NCI-3170 Phase I **FLAM** (n=34 AML, ALL, CML) (1st line/rel/refr AML n=26) dose escalation of alvocidib Bolus Accrued 3/2001-11/2003 Johns Hopkins (PI), Univ. MD, Baltimore VA Med Center, Mayo-Rochester	Bolus 1-hr IV /day x3 DL1: 40mg/m^2 DL2: 50mg/m^2 DL3: 60mg/m^2 Med. Age= 54	26 AML pts: DL1: 4 DL2: 19 DL3: 3	26 AML pts: 1st line 4/26 (15%) 1st rel. 3/26 (11%) 2nd rel. 4/26 (15%) Refractory 15/26 (58%)	26 AML pts: 7/26 (27%) prior MDS 2/26 (8%) t-AML	26 AML pts: 17 (65%)
STUDY 2: JHU-0254 / NCI-3170 Phase II **FLAM** extension; Poor-risk AML (N=62), 50mg/m^2 Bolus FLAM Accrued 1/2004-3/2006. Johns Hopkins	Bolus 1-hr IV /day x3 50mg/m^2 Med. Age= 58	62 AML pts	62 AML pts: 1st line 15/62 (24%) 1st rel. 24/62 (39%), CR1 9mos Refractory 23/62 (37%) (13 primary, 10 multiple)	62 AML pts: 1st line: 13 MDS/MPD & 2 t-AML Rel & Refr: 7/47 (15%) prior MDS 3/47 (6%) t-AML	
STUDY 3: OSU-0479 / NCI-6947 Phase I (rel/refr AML n=19; 5 ALL) Alvocidib *Monotherapy* Hybrid schedule dose escalation Accrued 4/2005-8/2007, OSU	Hybrid 30 min. IV & 4-hr Infusion/day x 3 DL1: 20/30 mg/m^2 DL2: 30/35 mg/m^2 DL3: 30/50 mg/m^2 DL4: 40/60 mg/m^2 (DL4 determined to be *monotherapy* MTD) DL5: 50/75 mg/m^2 Med. Age= 62	19 AML pts: DL1: 1 (refr) DL2: 5 (1 rel) DL3: 3 (2 rel) DL4: 8 (4 Rel) DL5: 2 (rel)	19 AML pts: Relapsed: 9/19 (47%) Refractory: 10/19 (53%)	19 AML pts: 5/19 (26%)	
STUDY 5: J06133 / NCI-7889 Phase I **FLAM** (rel/refr AML n=49) (+3 ALL, +3 t-myeloid ABL N=55) dose escalation of "*HYBRID*" alvocidib Accrued 5/2007–1/2009, Johns Hopkins	Hybrid 30 min. IV & 4-hr Infusion/day x 3 DL1: 20/30 mg/m^2 DL2: 25/35 mg/m^2 DL3: 30/40 mg/m^2 DL4: 30/50 mg/m^2 DL5: 30/60 mg/m^2 DL6: 30/70 mg/m^2 (DL6 = MTD) Med. Age= 62	49 AML pts: DL1: 5 DL2: 7 DL3: 5 DL4: 6 DL5: 24 (expanded) DL6: 2	49 AML pts: Relapsed: 12/49 (24%) Refractory: 37/49 (76%)	49 AML pts: R/R MPD-AML: 5/49 (10%)	49 AML pts: 37/49 (67%) 14/49 single cytogenetics 17/49 complex karyotype 7/49 (14%) FLT3+
STUDY 6: ECOG 1906 Phase II **FLAM** (Rel/Refr AML, FLAM n=36), 30/60mg/m^2 Hybrid alvocidib in FLAM. Mayo-Rochester (PI); Accrued 5/2007-8/2013 *(ASH Abstract #3742, Nov. 2014)*	36 AML/FLAM pts: 30/60 mg/m^2 Hybrid alvocidib in FLAM Med. Age= 62 (19-69)	36 AML/FLAM pts: one DL	36 AML/FLAM pts: relapsed <1 year after initial CR, or refractory to initial induction therapy (<2 courses) or to first re-induction after a relapse (<1 course)	36 AML/FLAM pts:	36 AML/FLAM pts:

Table 3: Alvocidib/FLAM in AML (Efficacy Parameters in Relapsed/Refractory Patients)

List of Studies	Efficacy Parameters					
	CR-Relapsed	RFS/EFS-Relapsed	OS- Relapsed	CR-Refractory	RFS/EFS- Refractory	OS- Refractory
STUDY 1: JHU-0254 / NCI-3170 Phase I **FLAM** (n=34 AML, ALL, CML) (1st line/rel/refr AML n=26) dose escalation of alvocidib bolus Accrued 3/2001-11/2003 Johns Hopkins (PI), Univ. MD, Baltimore VA Med Center, Mayo-Rochester	2/7 (29%)	*No RFS or survival data*		2/15 (13%)	*No RFS or survival data*	
STUDY 2: JHU-0254 / NCI-3170 Phase II **FLAM** extension; Poor-risk AML (N=62), 50mg/m² Bolus FLAM Accrued 1/2004-3/2006. Johns Hopkins	18/24 (75%) 5 of 18 relapsed pts then had BMT	DFS for 15 1st line + 24 Relapsed + 23 Refractory Pts: Among 32 CR pts: Median DFS=11 mos	OS for 15 1st line + 24 Relapsed + 23 Refractory Pts: Among 32 CR pts Median OS = 18 mos	2/13 (15%) Refr. & 0/10 Multiple Refr. 1 of 2 primary refractory pts then had BMT		
STUDY 3: OSU-0479 / NCI-6947 Phase I (rel/refr AML n=19; 5 ALL) Alvocidib *Monotherapy* *Hybrid* schedule dose escalation Accrued 4/2005-8/2007, OSU		*No RFS or survival data*		1/19 AML pts (5%) CRi (Primary refractory AML; DL4: 40/60)	*No RFS or survival data*	
Study 5: J06133 / NCI-7889 Phase I FLAM (rel/refr AML n=49) (+3 ALL, +3 t-myeloid ABL N=55) dose escalation of *Hybrid* alvocidib Accrued 5/2007–1/2009, Johns Hopkins	Relapsed AML: 11/12 (92%) 13/38 CRs in Adverse Cytogen. 2/7 CRs in FLT3+ patients 16/22 (73%) of CR patients (AML+ ALL+ ABL) had BMT	*DFS for all 49 AML +3 ALL+ 3 biphenotypic pts; relapsed +refractory:* For all CR patients, 19 AML, 1 ALL, 2 ABL median DFS was not yet reached: range 1.8-30 mos	*OS for all 49 AML +3 ALL+ 3 biphenotypic pts; relapsed + refractory:* Med. OS = 7.4 mos. For all CR patients, 19 AML, 1 ALL, 2 ABL median OS was not yet reached: range 3.7-31 mos	Primary refractory 5/16 (31%) Multi-refractory: 1/16 (6%) R/R MPD-AML : 2/5 (40%)		
STUDY 6: ECOG 1906: Phase II **FLAM** (Rel/Refr AML, FLAM n=36), 30/60mg/m² Hybrid alvocidib in FLAM. Mayo-Rochester (PI) Accrued 5/2007-8/2013	10/36 (28%) (6 CR + 4 CRi)	*No RFS or survival data*			*No RFS or survival data*	

Table 4: Alvocidib/FLAM in AML (Safety Data in Relapsed/Refractory Patients)

List of Studies	Safety Data (SAEs ≥Grade 3)						
	TLS	GI	Neutropenic Fever/Infection	Fatigue	Cardiac	Induction TRM	Other
STUDY 1: JHU-0254 / NCI-3170 Phase I **FLAM** (n=34 AML, ALL, CML) (1st line/rel/refr AML n=26) dose escalation of alvocidib Bolus dosing for DLT-PK determination Accrued 3/2001-11/2003 Johns Hopkins (PI), Univ. MD, Baltimore VA Med Center, Mayo-Rochester	9/34 (26%) Mild TLS no rel/refr AML pt required dialysis or had coagulopathy	3/34 (9%) diarrhea 3/34 (9%) oral mucositis, 3/34 (9%) GI mucositis	2/34 (6%) gr. 5 fungal infections		1 pt DL3 sudden death with Hx hypertensive CAD & DVT/emboli, 1pt at DL3 with extramed. Infiltrate developed decr. LVEF, acute cardiomyopathy and sudden death	4/34 (12%) 2 pts fungal sepsis, 2 cardiac-related	12/26 had 50% reduction in circ. blasts post-alvo.
STUDY 2: JHU-0254 / NCI-3170 Phase II **FLAM** extension; Poor-risk AML (N=62), 50mg/m^2 Bolus FLAM Accrued 1/2004-3/2006. Johns Hopkins	15/47 (32%) with 1 pt. requiring dialysis	1 gr. 3 GI mucositis			D 1-5 Alvo.: 2 gr.2 atrial arrhythmia D 6-9+, 1 gr.3 arrhythmia & 3 gr. 3 decr. LVEF	3 (5%) fungal infection & Multi-organ failure	2 ARDS with fungal pneumonia
STUDY 3: OSU-0479 / NCI-6947 Phase I (rel/refr AML n=19; 5 ALL) Alvocidib *Monotherapy* *Hybrid* schedule dose escalation Accrued 4/2005-8/2007, OSU	1 (5%) AML pt. required dialysis	Diarrhea (DLT) 7/24 (29%) Mucostis 1 (4%)	Neutropenic Fever/ Infection 14/24 (58%)	10/24 (42%)	3/24 (12.5%) Decreased EF, hypotension, prolonged QT	1/24 (4%) TLS followed by fungal sepsis	
STUDY 5: J06133 / NCI-7889 Phase I **FLAM** (rel/refr AML n=49) (+3 ALL, +3 t-myeloid ABL N=55) dose escalation of *Hybrid* alvocidib Accrued 5/2007–1/2009, Johns Hopkins	5/55 (9%) 1 t-ALL pt. required dialysis	Mucositis: oral 4/55 (7%) GI 2/55 (4%)	Infection 4/55 (7%)		3/55 (5%) Decreased EF, A-fib with rapid ventricular response, pericarditis	5/55 (9%) TRM-60 Infection, Multi-organ failure	6/55 (11%) Hyperbilirubinemia
STUDY 6: ECOG 1906: Phase II **FLAM** (Rel/Refr AML, FLAM n=36), 30/60 mg/m^2 Hybrid alvocidib in FLAM. Mayo-Rochester (PI) Accrued 5/2007-8/2013						10/36 (28%) 1st 27 FLAM pts: 5/6 deaths TRM-30 due to Septic shock & Multi-org. failure (5 pts >60)	

2.6.3 Newly Diagnosed High-Risk AML

Multiple phase II studies have been conducted using ACM (FLAM) in patients with newly diagnosed high-risk AML. In most studies high-risk AML was defined as disease that was treatment related, had secondary AML, or had adverse-risk cytogenetics. A key study was NCI-8972 (Table 1) where 165 newly diagnosed poor-risk patients were randomized to ACM versus 7&3. The primary endpoint of CR following one induction cycle was found to be statistically significant (p=0.08) and in favor of ACM-treated patients (70% CR) versus 7&3-treated patients (46% CR). ACM produced CR in 31/52 (60%) of patients with secondary AML compared to 9/26 (35%) treated with 7&3. In this study comparing ACM with 7&3 in poor-risk newly diagnosed patients the adverse events were similar in both arms. Comparative adverse events Grade ≥3 included (ACM:7&3): febrile neutropenia (48%, 45%), infection (35%, 38%), hepatic dysfunction (21%, 23%) and GI events (11%, 9%). Treatment related mortality was also similar between the arms at 30-days: ACM 5% versus 7&3 2% or at 60-days ACM 10% versus 7&3 4%. The majority of early deaths (8/11 patients) on ACM were >60 years.

2.7 Justification for Study Treatment Plan

Based on the initial data from single-arm Phase 1 and 2 studies as well as randomized Phase 2 studies, Tolero is pursuing regulatory approval for alvocidib as part of the alvocidib, cytarabine (ara-c) and mitoxantrone hydrochloride regimen or “ACM regimen” in patients with relapsed or refractory AML. As previously noted, because there is no standard care for patients with relapsed/refractory AML, the 2014 NCCN guidelines recommend that these patients enroll in a clinical study testing new drugs or new combinations of drugs. The combination of cytarabine and mitoxantrone (CM) is one of the regimens that can be considered for these patients.

NOXA binds and deactivates MCL-1. Early studies have shown that MCL-1 dependence can predict the clinical activity of alvocidib in AML patient samples and suggests an important role for MCL-1 activity in predicting alvocidib activity. As such, Tolero is conducting this Phase 2 study to test the ability of MCL-1 activity to predict which patients will response favorably to alvocidib treatment when administered as a bolus along with cytarabine and mitoxantrone.

While both the bolus as well as hybrid dosing regimens of FLAM have shown substantial activity in patients with AML, the hybrid regimen will be used in this study. Clinical data from a randomized study that compared the two dosing regimens suggest that the hybrid schedule tends to produce a higher remission rate (62% versus 74%) in poor-risk, newly diagnosed AML patients [17].

We have also observed a high CR rate (39%) with the hybrid regimen in relapsed and refractory AML patients (and 92% CR among relapsed-only patients) [10]. In addition, the safety profile of the two regimens appears similar, though there may be a trend to lower early mortality with the hybrid regimen. In these two studies, the incidence of Tumor Lysis Syndrome (TLS) was 9%, and treatment-related mortality was 8% and 9%, respectively.

Dosing for the TPI-ALV-201 study is based on the first Phase 1 AML, dose-escalation study of alvocidib and ara-C + mitoxantrone conducted at the University of Maryland and Johns Hopkins University (Study JHOC J0254/NCI-3170 [12]). This initial study administered alvocidib by 1-hour bolus infusion daily × 3 at doses of 40, 50 or 60 mg/m^2/d, followed by 2 gm/m^2/72h ara-C over Days 6-8 and 40 mg/m^2 mitoxantrone bolus on Day 9, in 34 adults with poor-risk, acute leukemia (including 22 with relapsed/refractory AML and 4 first-line AML patients). Additional studies utilizing a pharmacologically modeled 'hybrid' schedule of alvocidib—a shorter, 30-minute IV bolus of approximately one-third to one-half the total dose followed by a 4-hour continuous infusion of the remainder of the dose [19]—have been shown to be effective with acceptable safety mitigated by aggressive prophylactic treatments [10, 14, 17]. As such, the alvocidib dosing regimen has been revised in subsequent studies to a hybrid schedule of a 30-minute IV infusion followed by a 4-hour infusion which is the regimen chosen for this Phase 2 study.

The primary change being implemented in Amendment 11 is modifying the lower limit of MCL-1 dependency required for inclusion in the study from ≥40% to ≥30%. At the time that this biomarker-driven study was conceived, the study design with hypothesis being tested was highly innovative with the use of an MCL-1 dependence assay to try and identify which patients would be most likely to respond to alvocidib therapy. As the trial progressed, a second generation assay was developed which has shown more consistency and reliability than the first generation assay. More recent results indicate that an MCL-1 dependence score of 30 is the appropriate clinical cutoff for the assay versus an MCL-1 dependence score of 40 that was used previously. Upon review of data from the Exploratory Arm that included patients with MCL-1 dependence scores of 30-39%, it became apparent that both the response and 1-year survival rates were similar to patients in the >40% MCL-1 dependence group, ie, 62% vs 52% and 66% vs 47%, respectively. In addition, the inclusion of patients with MCL-1 dependence scores of ≥30-39% could increase accrual by 25-30%, thereby allowing for more rapid completion of the current study and more timely development of the next generation of trials leading to registration. Moreover, inclusion of the ≥30-39 MCL-1 dependence group may be more accurate in terms of serving the subpopulation of patients with relapsed/refractory AML who are potentially responsive to, and likely to benefit from, the ACM regimen.

2.8 Summary of Risk and Benefits

The safety profile for ACM (FLAM) has been well-described in the eight clinical studies in patients with AML, and appears to be acceptable for these poor-risk and pretreated patient populations. The early observation of tumor lysis and the potential for renal failure has resulted in an aggressive prophylaxis approach to manage the dramatic lysis of leukemic blasts caused by FLAM. Treatment-related mortality (TRM) for patients treated with alvocidib/FLAM is approximately 8-10%, with a range up to 28% in Study 6 of relapsed/refractory AML patients (particularly among patients over 60 years old). Overall, TRM of alvocidib/FLAM appears to be comparable to other therapeutic options including 7&3 and intermediate- and high-dose cytarabine.

3. STUDY OBJECTIVES

Stage 1: Primary Objective

- To determine the CR rate in patients with relapsed or refractory AML with MCL-1 dependence of ≥***30***%

Stage 2: Primary Objective

- To compare CR rates between patients with relapsed or refractory AML with MCL-1 dependence of ≥***30***% receiving 1 cycle of ACM treatment and those receiving 1 cycle of CM.

Exploratory Objective (Stage 2): To determine if treatment with ACM can induce CR in patients with relapsed or refractory AML with MCL-1 dependence of ≥***30***% who failed to achieve CR following 1 cycle of CM.

4. INVESTIGATIONAL PLAN

"FLAM" (alvocidib, cytarabine, mitoxantrone hydrochloride) will now be referred to as "ACM".

"AM" (cytarabine, mitoxantrone hydrochloride) will now be referred to as "CM".

4.1 Overall Study Design

This is a Phase 2, open-label, randomized, multicenter, two-stage study to determine if MCL-1 dependence demonstrated by mitochondrial profiling in bone marrow samples will increase the Complete Remission (CR) rate after ACM treatment.

Stage 1 of the study will be single arm (ACM), conducted similarly to the first stage of a Simon 2-stage design. Patients with relapsed or refractory AML with demonstrated MCL-1 dependence of ≥***30***% by mitochondrial profiling in bone marrow will be screened according to study inclusion and exclusion criteria up to 14 days before the initiation of ACM study treatment. Once ≥13 patients enrolled in Stage 1 exhibit a CR, CRi, or CRp, enrollment into Stage 1 will close and additional patients will be enrolled in Stage 2 of the study.

Enrollment into Stage 1 will be limited to no more than 23 evaluable patients, however enrollment into Stage 2 may be initiated at any point after confirming CR, CRi or CRp responses in 13 Stage-1 patients.

In Stage 2, patients with relapsed or refractory AML with demonstrated MCL-1 dependence of ≥***30***% by mitochondrial profiling in bone marrow will be randomized 1:1 to receive either ACM or CM. Patients will be screened for study inclusion and exclusion criteria up to 14 days before the initiation of study treatment.

Response assessments in Stage 1 are defined by the International Working Group Criteria [20] and 2010 European LeukemiaNet (ELN) [21]. The 2017 ELN criteria will be used to determine patient responses during Stage 2. Response assessments *(Appendix E)* will include:

- CR rate = Percentage of patients achieving CR
- Combined CR Rate = Percentage of patients achieving:
  - CR = Bone marrow blasts <5%; absence of blasts with Auer rods; absence of extramedullary disease; hematologic recovery (absolute neutrophil count [ANC] >1000 µL and platelet count >100,000 µL); independence of red cell transfusions; *plus*
  - CRi = Meets all CR criteria except for residual neutropenia (ANC <1000 µL) or lack of recovery of any other hematopoietic cell; *plus*
  - CRp = Meets all CR criteria except for residual thrombocytopenia (platelet count <100,000 µL) †

- Combined Response Rate = Percentage of patients achieving:
  - CR
  - CRi
  - CRp †
  - PR = Meets all hematologic values required for CR but with a decrease of at least 50% in the percentage of blasts to ≥5% to ≤25% in bone marrow
- Overall Survival (OS) = Time from randomization (Day 1) until death from any cause
- Event-free Survival (EFS) = Time from randomization (Day 1) until (a) treatment failure, (b) relapse after CR, or (c) death from any cause, whichever occurs first, censored at 2 years
- Rate of Stem Cell Transplantation = Percentage of patients proceeding directly to stem cell transplantation

†Note that CRp has been retained in definitions for Combined CR Rate and Combined Remission Rate because Stage-1 patients were assessed using the IWG criteria. CRp will not be used for assessing response in Stage-2 patients and, therefore, will not be included in the definitions of Combined CR Rate and Combined Remission Rate in Stage 2.

The CR rate in patients failing 1 cycle of CM and crossing over to receive ACM will also be determined.

Safety assessments will include:

- Mortality from any cause at 30 and 60 days
- Adverse events graded according to National Cancer Institute's Common Terminology Criteria for Adverse Events (NCI CTCAE), version 4.03 in Stage 1 and version 5.0 in Stage 2
  - The multivariate analysis and risk score prediction model by Montesinos and colleagues [22] will be used to assess the potential for development of TLS (*Appendix F)*

Treatment assessments will include:

- Bone marrow biopsies and/or aspirates will be performed at hematologic recovery (ie, absolute neutrophil count (ANC) >1000 µL and platelet count >100,000 µL) or Day 45, whichever occurs first. Slides from bone marrow will also be collected.
- Complete blood counts and chemistries assessed daily while hospitalized for chemotherapy administration and weekly thereafter

- Overall survival (OS) monitored monthly during year 1, every 2 months during year 2

- Intensive monitoring of renal function, electrolytes and uric acid levels

Pharmacodynamic assessments will include:

- Determination of MCL-1 dependence at prescreening using bone marrow aspirate

Patients who achieve CR, CRi, CRp, or PR after the first cycle (completion of all doses) may receive up to 3 additional optional cycles of treatment.

- After completing the first cycle of treatment, continued use of mitoxantrone is optional.
- Mitoxantrone must be omitted from subsequent cycles if the patient's lifetime daunorubicin equivalent exceeds 460 mg/m^2 (see *Appendix G* for conversion table) or the LVEF drops below 45%.

Patients not demonstrating evidence of CR, CRi, CRp, or PR after the first cycle of treatment will be considered for removal from the study, although with permission of the Medical Monitor, induction treatment may continue, if clinically indicated, and provided there is no evidence of toxicity ≥NCI CTCAE grade 4 (v4.03 in Stage 1, v5.0 in Stage 2; see *Appendix C*). This study also includes a crossover option for those randomized to CM (see below).

- Crossover: Patients randomized to CM with progressive disease or no response after 1 cycle of CM may cross over to receive ACM. In addition, patients randomized to CM with the best response of a PR after 2 cycles of CM may also cross over to receive ACM. Patients who cross over to ACM may receive up to a combined total (CM + ACM) of 4 cycles. Patients crossing over who meet the above criteria (lifetime daunorubicin equivalent exceeds 460 mg/m^2 or the LVEF drops below 45%) will receive only AC (alvocidib and cytarabine) for all cycles instead of ACM.

Complete remission rates will be compared across treatment groups using the Cochran-Mantel-Haenszel general association test stratified by response to most recent induction therapy. The same test will be applied to assess the statistical significance of the secondary response rate endpoints.

Incidence rates of treatment-emergent adverse events (TEAEs) will be summarized within treatment group at the Medical Dictionary for Regulatory Activities (MedDRA) preferred term and primary system organ class levels. Similar summaries will be made for subsets of adverse events (AEs) such as (1) those judged by the Investigator to be related to study treatment, and (2) serious adverse events (SAEs).

Other routine safety assessments (eg, clinical laboratory parameters and vital signs) will be summarized by treatment group using mean, standard deviation, median, minimum and maximum changes from baseline values.

The study is expected to take 20-24 months to enroll up to 137 patients: 25 patients in Stage 1; 56 patients in the 4 Exploratory Arms (closed after Amendment 8); and 56 patients (28 patients in each of two treatment arms) in Stage 2.

4.2 Randomization Criteria

This study follows a randomized, two-stage design. During Stage 1, patients will receive ACM:

- **ACM** – **A**lvocidib, **C**ytarabine (ara-c), and **M**itoxantrone).

Once a minimum response threshold has been met in Stage 1 as defined in the Statistical Analysis Plan (SAP), Stage 1 will be closed to enrollment and new patients will be enrolled into Stage 2 and randomized 1:1 to receive either ACM or CM:

- **CM** – **C**ytarabine (ara-c) and **M**itoxantrone

Randomization to treatment arm will be stratified by:

- Response to first-line therapy:
  - Refractory: persistent disease or complete remission [CR] duration <90 days
  - Early relapse: CR duration 90 days to 1 year
  - Late relapse: CR duration >1 year but <24 months

4.3 Controls

An active comparator will be used (treatment with cytarabine and mitoxantrone [CM regimen]).

4.4 Patient Population

4.4.1 Number of Patients

A sufficient number of patients with demonstrated MCL-1 dependence by mitochondrial profiling in bone marrow will be screened in order to obtain up to 137 eligible and evaluable patients. This includes 25 patients with MCL-1 dependence ≥***30***% evaluated for response in Stage 1; 56 patients in the 4 Exploratory Arms (closed after Amendment 8); and 56 patients with MCL-1 dependence ≥***30***% (28 patients in each of the two treatment arms) in Stage 2.

Patients who drop out prior to dosing will be replaced, but will still be included in the Intent-to-treat (ITT) analysis population. Patients who drop out of Stage 1 prior to being assessed for response will be replaced, but will still be included in the ITT analysis population and in the Safety analysis population according to the analysis populations defined in *Section 11.3*.

4.4.2 Inclusion Criteria

To be eligible for participation in the study, patients must meet all of the following inclusion criteria:

During Prescreening:

1. Be between the ages of ≥18 and ≤65 years
2. Have an established, pathologically confirmed diagnoses of AML by World Health Organization (WHO) criteria excluding acute promyelocytic leukemia (APL-M3) with a bone marrow of >5% blasts based on histology or flow cytometry
3. Be in first relapse (within 24 months of CR) or have failed induction therapy* (no CR or CRi after treatment with an intensive regimen [eg, anthracycline/cytarabine ± etoposide, gemtuzumab ozogamicin, or cladribine])
   *Induction therapy may involve 1 or 2 cycles of the same regimen. Efficacy assessment of induction therapy must be >21 days from the start of the previous induction cycle.
4. Demonstrate MCL-1 dependence of ≥***30***% by mitochondrial profiling in bone marrow

During Screening:

5. Have an Eastern Cooperative Oncology Group (ECOG) performance status (PS) ≤2
6. Have a serum creatinine level ≤1.8 mg/dL
7. Have an alanine aminotransferase (ALT)/aspartate aminotransferase (AST) level ≤5 times upper limit of normal (ULN)
8. Have a total bilirubin level ≤2.0 mg/dL (unless secondary to Gilbert syndrome, hemolysis, or leukemia)
9. Have a left ventricular ejection fraction (LVEF) >45% by echocardiogram (ECHO) or multigated acquisition (MUGA) scan
10. Be nonfertile or agree to use an adequate method of contraception. Sexually active patients and their partners must use an effective method of contraception associated with a low failure rate during and for at least 6 months after completion of study therapy (see *Section 4.7.3*).

11. Be able to comply with the requirements of the entire study.
12. Provide written informed consent prior to any study related procedure. (In the event that the patient is re-screened for study participation or a protocol amendment alters the care of an ongoing patient, a new informed consent form must be signed.)

4.4.3 Exclusion Criteria

Patients meeting any one of these exclusion criteria will be prohibited from participating in this study.

1. Received more than 2 cycles of induction therapy for AML. Investigational agents as part of front-line therapy for AML may by acceptable following discussion with the Medical Monitor. Hydroxyurea is permitted (see #5 below).
2. Received any previous treatment with alvocidib or any other CDK inhibitor
3. Received a hematopoietic stem cell transplant within the previous 2 months
4. Have clinically significant graft versus host disease (GVHD), or GVHD requiring initiation or escalation of treatment within the last 21 days
5. Require concomitant chemotherapy, radiation therapy, or immunotherapy. Hydroxyurea is allowed up to the evening before starting (but not within 12 hours) of starting treatment on either arm.
6. Received >360 mg/m^2 equivalents of daunorubicin (see *Appendix G* for conversion table)
7. Have a peripheral blast count of >30,000/mm^3 (may use hydroxyurea as in #5 above)
8. Received antileukemic therapy within the last 3 weeks (with the exception of hydroxyurea or if the patient has definite refractory disease). Refractory patients who received therapy within the last 3 weeks may be eligible with prior approval of the Medical Monitor.
9. Diagnosed with acute promyelocytic leukemia (APL, M3)
10. Have active central nervous system (CNS) leukemia
11. Have evidence of uncontrolled disseminated intravascular coagulation
12. Have an active, uncontrolled infection
13. Have other life-threatening illness
14. Have other active malignancies or diagnosed with other malignancies within the last 6 months, except nonmelanoma skin cancer or cervical intraepithelial neoplasia

15. Have mental deficits and/or psychiatric history that may compromise the ability to give written informed consent or to comply with the study protocol.
16. Are pregnant and/or nursing
17. Have received any live vaccine within 14 days prior to first study drug administration

4.5 Study Treatments

4.5.1 Calculation of Dose

The dosage of study drugs will be recalculated at the beginning of each new treatment cycle to reflect changes in the body surface area (BSA) that may have occurred but will remain the same for all treatments within a treatment cycle.

4.5.2 Treatment Plan

This is an open-label, randomized, multicenter, two-stage study in patients with AML with demonstrated MCL-1 dependence of ≥***30***% by mitochondrial profiling in bone marrow who are either in first relapse or have primary refractory AML.

Stage 1 of the study will be single arm and all enrolled in this arm will receive treatment with ACM. Stage 2 will be two-arm; patients enrolled in this stage will be randomized to receive treatment with either ACM or CM.

4.5.3 Treatment Schema

4.5.3.1 Stage 1: Treatment with ACM over Days 1-9

Patients enrolled in Stage 1 will receive treatment with ACM as outlined below:

- **Days 1, 2 and 3: alvocidib (*A*) - Administer Daily**
  30 mg/m^2 as a 30-minute (±10 minutes) intravenous (IV) bolus
  followed by
  60 mg/m^2 over 4 hours (±15 minutes) as an IV infusion
- **Days 4 and 5: Rest (no chemotherapy treatment)**
- **Days 6, 7, and 8: cytarabine (*C*) - Continuous over 72 hours**
  2 gm/m^2 by continuous IV infusion over 72 hours (±2.5 hours)
  (ie, 667 mg/m^2 daily for total of 2 gm/m^2)
- **Day 9: mitoxantrone hydrochloride (M) is administered 12 hours after completion of cytarabine treatment**
  40 mg/m^2 by IV infusion over 1-2 hours (±10 minutes)

4.5.3.2 Stage 2: Treatment with ACM or CM

Patients enrolled in Stage 2 will be randomized to either ACM or CM and will receive treatment as outlined below:

Stage 2: Randomized to ACM: Treatment with ACM over Days 1-9

Follow the Stage 1 treatment schema above

Stage 2: Randomized to CM: Treatment with CM over Days 1-4

- **Days 1, 2, and 3: cytarabine (C) - Continuous over 72 hours**

  2 gm/m^2 by continuous IV infusion over 72 hours (±2.5 hours)
  (ie, 667 mg/m^2 daily for total of 2 gm/m^2)

- **Day 4: mitoxantrone hydrochloride (M) is administered 12 hours after completion of cytarabine treatment**

  40 mg/m^2 by IV infusion over 1-2 hours (±10 minutes)

4.5.3.3 Evaluation After Cycle 1

- Patients who achieve CR, CRi, CRp, or PR after the first cycle (completion of all doses) may receive up to 3 additional optional cycles of treatment.
  - After completing the first cycle of treatment, continued use of mitoxantrone is optional.
  - Mitoxantrone must be omitted from subsequent cycles if the patient's lifetime daunorubicin equivalent exceeds 460 mg/m^2 (see *Appendix G* for conversion table) or the left ventricular ejection fraction (LVEF) drops below 45%.
- Patients not demonstrating evidence of CR, CRi, CRp, or PR after the first cycle of treatment will be considered for removal from the study, although with permission of the Medical Monitor, induction treatment may continue if clinically indicated and provided there is no evidence of toxicity ≥NCI CTCAE grade 4.
- Perform the End of Study Assessments for those patients who do not continue on study to receive at least one additional cycle of treatment (see *Section 5.5*).

4.5.3.4 Crossover (Stage 2)

- Patients randomized to CM with progressive disease or no response after one cycle of CM may cross over to receive ACM.
- Patients randomized to CM with a best response of PR after 2 cycles of CM may also cross over to receive ACM.

- Continued use of mitoxantrone is optional after completion of one cycle of ACM
- Mitoxantrone must be omitted from subsequent cycles if the patient's lifetime daunorubicin equivalent exceeds 460 mg/m^2 (see *Appendix G* for conversion table) or the left ventricular ejection fraction (LVEF) drops below 45%. Patients crossing over who meet the above criteria will receive only AC (alvocidib and cytarabine) for all cycles instead of ACM.
- Patients who cross over to ACM may receive up to a combined total (CM + ACM) of 4 cycles.

4.6 Management of Toxicities and Dosage Modifications

Suggested doses of supportive care therapies are provided; however, adjustment of the dosages based on the patient's clinical condition or each institution's standard of care is permitted.

4.6.1 Management of Nonhematologic Toxicities

Adverse events may be treated with concomitant medications, as deemed clinically indicated by the Principal Investigator (PI). All concomitant medications must be recorded in the source and on the appropriate case report form (CRF).

Adverse events that are moderate to severe in intensity (see *Section 8.1* and *Appendix C* for toxicity grading) and considered possibly or probably related to study drug treatments may result in the delay or termination of study treatment in affected patients.

4.6.1.1 Hyperkalemia and Tumor Lysis Syndrome

Tumor lysis may occur as part of initial cytoreductive therapy. The most extreme form, known as Tumor Lysis Syndrome (TLS), is characterized by hyperkalemia, hyperuricemia, hyperphosphatemia, increased lactate dehydrogenase (LDH), coagulopathy, and a potential cytokine release syndrome.

Mandatory IV hydration with 0.45% NaCl (or similar hydration fluid per institutional standard) sterile solution at 100 cc/hour for at least 10 hours prior to initiation of the first dose of chemotherapy during Cycle 1 (optional for subsequent cycles) in all treatment arms. If, by Day 4, there is no evidence of tumor lysis syndrome, the hydration rate can be reduced to a maintenance level.

Diligent monitoring of urine output **frequently** to ensure that fluid output equals fluid input. If input is greater than output by 10%, administration of diuretics is encouraged. **Replacement of excessive fluid losses, including from diarrhea, should be done unless otherwise clinically indicated.**

- Mandatory allopurinol orally each day of dosing for first cycle to be started at same time as initiation of IV hydration in all treatment arms.

- Mandatory oral phosphate binder to be started at the same time as initiation of IV hydration in all treatment arms, unless contraindicated.
- Evaluation of laboratory indicators of tumor lysis syndrome (TLS)
    - Tumor lysis laboratory evaluations ('tumor lysis labs') include electrolytes (sodium, potassium, chloride, and carbon dioxide) as well as creatinine, calcium, lactate dehydrogenase (LDH), uric acid, and phosphorus levels.
    - For patients **at high risk** for TLS including those with risk scores of ≥2 according to the table in *Appendix F*, those with monocytic leukemia phenotype, or those with a history of FLT3 positive AML:
        - Patients randomized to ACM (or who cross over to ACM after failure of CM):
            - Obtain a STAT serum potassium at end of alvocidib infusion
            - Strongly recommend serum potassium at 2 hours post end of alvocidib infusion as best practice
            - Obtain full TLS panel at 4 hours post end of alvocidib infusion
            - If evidence of clinically meaningful TLS, obtain TLS panel every 2 hours during the first 24 hours post end of alvocidib infusion (LDH levels are recommended to be assessed at least once every 24 hours).
            - If no evidence of clinically meaningful TLS, obtain TLS panel every 4 hours during the first 24 hours post end of alvocidib infusion (LDH levels are recommended to be assessed at least once every 24 hours).
            - If, after the first 24 hours post end of alvocidib infusion, there is no evidence of TLS, obtain TLS panel approximately every 6 hours for the remainder of alvocidib treatment and then every 12 hours after completion of alvocidib and during cytarabine and mitoxantrone (LDH levels are recommended to be assessed at least once every 24 hours).
            - Monitor fibrinogen levels at baseline and then as clinically indicated.
            - Patients who are determined to be at intermediate- or high-risk for TLS should be considered for rasburicase prophylaxis according to institutional standards

- *Patients randomized to* CM:
  - During the first 24 hours - Monitor tumor lysis labs at the start of cytarabine and approximately every 6 hours. Laboratory studies during this period should be run as a “STAT” to ensure the results are available in a timely manner.
  - If no evidence of TLS during the first 24 hours, then monitor tumor lysis labs every 12 hours until completion of mitoxantrone.
  - Monitor fibrinogen levels at baseline and then as clinically indicated.

- For patients **NOT at high** risk for TLS:
  - *Patients randomized to ACM (or who cross over to ACM after failure of CM):*
    - During the first 24 hours - Monitor tumor lysis labs at the start of alvocidib and approximately every 4 hours. Laboratory studies during this period should be run as a “STAT” to ensure the results are available in a timely manner (LDH levels are recommended to be assessed at least once every 24 hours).
    - If no evidence of TLS during first 24 hours, then monitor tumor lysis labs approximately every 6 hours for the remainder of alvocidib treatment and then every 12 hours after the completion of alvocidib and during cytarabine and mitoxantrone (LDH levels are recommended to be assessed at least once every 24 hours).
    - Monitor fibrinogen levels at baseline and then as clinically indicated.
  - *Patients randomized to CM*:
    - During the first 24 hours - Monitor tumor lysis labs at the start of cytarabine and approximately every 6 hours. Laboratory studies during this period should be run as a “STAT” to ensure the results are available in a timely manner.

- If no evidence of TLS during first 24 hours, then monitor tumor lysis labs every 12 hours until completion of mitoxantrone.
- Monitor fibrinogen levels at baseline and then as clinically indicated.

Risk of TLS and Guidelines for Management of High Risk Patients

TLS management during treatment with alvocidib was implemented in previous studies, which included medical prophylaxis for hyperuricemia, as well as aggressive monitoring and management of hyperkalemia and other biochemical laboratory abnormalities. Rapid development of hyperkalemia has been of particular concern in earlier studies. While these guidelines are not necessarily consistent with specific standard recommendations for the treatment of TLS, they are recommended based on previous experience with the treatment of patients with alvocidib. These measures resulted in a lower incidence of TLS without adverse outcomes.

For this reason, you are encouraged to follow the recommended guidelines below but may follow your own institution's protocols in determining the best treatment for your patients.

- If potassium levels are increasing to >4.0 mEq/L, patients should receive a 30-gm dose of sodium polystyrene sulfonate, unless there are other likely causes of hyperkalemia other than TLS or contraindication to its use.
- If potassium levels rise to >5.0 mEq/L, in addition to the 30-gm dose of sodium polystyrene sulfonate, patients should also receive 10 units of IV rapid-acting insulin and 25 gm (one ampule) of IV dextrose 50%, unless there are other likely causes of hyperkalemia other than TLS or contraindication to its use.
- If potassium levels rise to >5.5 mEq/L, patients should be considered for emergent intermittent or continuous dialysis.
- Calcium supplementation should only be given for symptomatic hypocalcemia in this setting to avoid renal precipitation of calcium phosphate crystals.
- Patients who develop clinical evidence of cytokine release syndrome or who have hyperkalemia requiring dialysis will receive immediate steroid therapy with an equivalent of at least 20 mg of IV dexamethasone.

4.6.1.2 Diarrhea

If diarrhea occurs during therapy, patients should initiate loperamide (or equivalent) 2 mg by mouth every 2 hours during the waking hours. The rapid introduction of loperamide (or equivalent) at the first signs of diarrhea is strongly encouraged. Testing for *Clostridioides difficile* (*C. difficile*) should start with first episode of diarrhea. Should testing indicate the presence of *C. difficile*, appropriate antibiotics targeting this infection should be initiated. Should testing exclude the presence of *C. difficile*, loperamide should be continued. Once the diarrhea is controlled, the time interval of loperamide may be titrated to a frequency that adequately controls the diarrhea. The diarrhea observed with alvocidib almost always resolves following completion of therapy, so treatment with loperamide following completion of therapy will not be required in most patients. If loperamide (or equivalent) does not control diarrhea, cholestyramine (or equivalent) 5 gm orally four (4) times daily may be added. For patients developing diarrhea during alvocidib administration, subsequent treatments should include a similar diarrhea prophylaxis. If diarrhea is not controlled with the above prophylactic regimen and is grade 2 or greater, therapy should be held until diarrhea has resolved. **Replacement of excessive fluid losses should be done unless otherwise clinically indicated.**

4.6.1.3 Nausea/Vomiting

Antiemetics (ie, 5-hydroxytryptamine [5-HT3] receptor inhibitor or other antiemetic medications) are permitted according to standard practices at each investigational site.

4.6.1.4 Infection Prevention

Prophylactic antibiotics including levofloxacin (or equivalent) 500 mg orally once daily and valacyclovir (or equivalent) 500 mg orally BID each day should be administered to patients in all treatment arms at the start of chemotherapy. Alternative prophylactic antibiotic or antiviral therapy is left to the discretion of the treating physician and according to institutional standards. For guidance on possible *C. difficile* infections, refer to *Section 4.6.1.2*.

Antifungal prophylaxis to be administered according to each institution's standard of care.

To prevent cytarabine-related conjunctivitis, corticosteroid eye drops per institutional formulary (eg, prednisolone 0.1%) must begin 1 day prior to institution of cytarabine infusion (ie, Day 6 for ACM; Day 1 for CM) and continue for at least 7 days.

Routine growth factor support is not allowed ≤Day 35 of therapy. Growth factor support can be given at the discretion of the Investigator >Day 35 of therapy in the presence of life-threatening infection with ongoing neutropenia.

Donor lymphocyte infusions are not allowed unless discussed and approved by the medical monitor.

4.6.2 Management of Hematologic Toxicities

Adverse events that are moderate to severe in intensity (see *Section 8.1* and *Appendix C* for toxicity grading) and considered possibly or probably related to study drug treatments may result in the termination of study treatment in the affected study patient. Such termination should be reviewed with the Sponsor's Medical Monitor at the earliest possible time (see *Section 8.5*). Following review with the Sponsor's Medical Monitor, the study patient may be permanently withdrawn from the study depending upon the nature and severity of the event.

Adverse events may be treated with concomitant medications, as deemed clinically indicated by the PI. All concomitant medications must be recorded on the appropriate CRF.

4.6.3 Dose Modifications

There will be no dose reductions for alvocidib or cytarabine. The dose of mitoxantrone must be reduced by 50% if the patient's total bilirubin value is >3 mg/dL and <5 mg/dL, and must be omitted if the patient's total bilirubin value is ≥5 mg/dL on the day of planned mitoxantrone administration. After completing the first cycle of treatment, continued use of mitoxantrone is optional. Mitoxantrone must be omitted from subsequent cycles if the patient's lifetime daunorubicin equivalent exceeds 460 mg/m^2 (see *Appendix G* for conversion table) or the left ventricular ejection fraction (LVEF) drops below 45%.

4.7 Concomitant Medications and Therapies

4.7.1 Previous Therapies

During Screening, patients will be asked about all previous therapies and all medications used during the previous 30 days from anticipated first dose. This information will be recorded in the source documentation and appropriate CRF along with the diagnosis or reason for use. If a branded product is being taken, the generic name should be reported, if known.

Subjects will not be enrolled into the study if they have had any previous treatment with alvocidib or other CDK inhibitor, or have received >360 mg/m^2 equivalents of daunorubicin (see *Appendix G* for conversion table).

4.7.2 Concomitant Therapies

Concomitant therapies are any new or existing medications or therapy taken by the patient including:

- Drugs, including but not limited to, prescription, over-the-counter, birth control pills/patches/hormonal devices, and homeopathic preparations
- Nondrug therapies, including but not limited to, thermal/laser/radiation procedures, vitamins, herbal medicines/supplements.

During the Screening process (up to two weeks prior to anticipated first dose of study drug), information on all concomitant therapies, medications, and procedures will be recorded in the source documents and appropriate CRF along with the diagnosis or reason for use.

Once the patient receives the first dose of study drug, recording of concomitant therapies will be limited to any new medication or modification of an existing medication taken for treatment of an adverse event (AE). These therapies will be recorded in the source documents and appropriate CRF along with the diagnosis or reason for use. Those therapies used for the treatment of an adverse event are to be linked to an AE and documentation of the AE must also be completed (refer to *Section 8*).

If a branded product is being taken, the generic name should be reported, if known.

4.7.2.1 Mandated / Permitted Therapies

Concomitant medications necessary for the health and well-being of the patient and that do not interfere with study assessments are permitted during the study at the Investigator's discretion. This includes the use of appropriate medications for the treatment of AEs and/or concurrent illnesses under the direction of the Principal Investigator. All such therapies must be recorded in the source and on the appropriate CRF.

In patients with rapidly proliferating disease, hydroxyurea may be administered up to the evening before starting treatment in either Stage, but not within 12 hours prior to dosing.

Patients may be allowed on study if they have undergone a diagnostic lumbar puncture (LP) for suspected CNS leukemic involvement. If, at the time of the LP, they received a single dose of intrathecal (IT) chemotherapy, that patient would still be considered eligible for this study so long as the LP was negative for CNS disease. Should the LP indicate positive cytology necessitating continued IT therapy, the patient would be ineligible for study inclusion.

Medications and procedures that are mandatory or permitted during the study are listed in *Section 4.6.1*.

4.7.2.2 Prohibited Therapies

The following medications are excluded from concomitant use:

- Antileukemic therapy (chemotherapy, radiation therapy, immunotherapy) within the last 3 weeks prior to the first study drug administration and during the cycle of study treatment (see *Section 4.7.2.1* for permitting prior hydroxyurea and IT chemotherapy)
- Live vaccines within 14 days prior to first study drug administration, during the study, and for approximately 3 months after the last dose of study drug.
- Donor lymphocyte infusions are not allowed unless discussed and approved by the medical monitor.

4.7.3 Birth Control Requirements for Fertile Patients

Sexually active patients and their partners must use an effective method of contraception associated with a low failure rate during and for at least 6 months after completion of study therapy. The following are considered effective contraceptives: (1) oral contraceptive pill; (2) condom plus spermicide; (3) diaphragm plus spermicide; (4) true abstinence when this is in line with the preferred and usual lifestyle of the subject (periodic abstinence [eg, calendar, ovulation, symptothermal, post-ovulation methods, etc], declaration of abstinence for the duration of exposure to study drugs, and withdrawal are not acceptable methods of contraception); (5) patient or partner surgically sterile; (6) patient or partner more than 2 years post-menopausal; or (7) injectable or implantable agent/device. Male patients enrolled in the study must use a condom to avoid exposing partners to semen, which may contain toxic drugs.

4.8 Protocol Deviations

It is expected that this study will be conducted as described in this protocol, except for an emergency situation in which the protection, safety and well-being of the patient requires immediate intervention, based on the judgment of the Principal Investigator (or a responsible, appropriately trained and credentialed professional[s] designated by the PI). In the event of a significant deviation from the protocol due to an emergency, accident or error, the PI or Designee must contact the Sponsor at the earliest possible time by telephone. This will allow an early joint decision to be made as to whether or not the patient should continue in the study. This decision will be documented in writing by both the PI and the Sponsor.

4.9 Other Precautions

Dose adjustments for nonhematologic and hematologic toxicities and laboratory abnormalities will be made according to *Section 4.6.1* and *Section 4.6.2*, respectively.

5. ON-STUDY CLINICAL AND LABORATORY EVALUATIONS

See *Appendix A* - Schedule of Activities

5.1 Prescreening (Stage 1, Stage 2)

- Obtain written informed consent (must be obtained prior to prescreening evaluations)
- Perform bone marrow aspiration for mitochondrial profiling to determine MCL-1 dependence. If the initial bone marrow aspirate is nonproductive or not diagnostic, the procedure must be repeated. (An aspirate must be obtained for mitochondrial profiling to determine MCL-1 dependence).
- Collect a disease history including pathological confirmed diagnosis of AML by WHO criteria and confirmation of either relapsed/refractory disease or newly diagnosed high-risk disease.

5.2 Screening (Stage 1, Stage 2)

5.2.1 Within 2 Weeks Prior to First Dose

If the MCL-1 dependence scores are ≥***30***%, perform the following activities and evaluations within 2 weeks prior to administration of the first dose of chemotherapy to determine if a patient meets the eligibility criteria:

- Collect and document a complete medical history including pathological confirmed diagnosis of AML by WHO criteria and all other measures of disease and disease symptoms (eg, extramedullary disease)
- Perform a complete physical examination including height (cm) and weight (kg)
- Assess ECOG PS (*Appendix B*)
- Record vital signs (temperature, heart rate, systolic and diastolic blood pressures)
- Perform bone marrow biopsy and/or aspiration for diagnosis and cytogenetic profiling. If the initial bone marrow biopsy and/or aspirate is nonproductive or not diagnostic, the procedure must be repeated.
    - If an aspirate with cytogenetics was performed as part of the mitochondrial profiling to determine MCL-1 dependence and it was done within 2 weeks of the first dose, then it does not need to be repeated.

- In addition, 6 to 8 bone marrow slides will be prepared at screening (in addition to the fresh bone marrow samples required for mitochondrial profiling to determine MCL-1 dependence) and sent to the Sponsor. Details regarding the collection and shipping of these slides is provided in the Study Manual.

- Perform 12-lead electrocardiogram (ECG)
- Perform echocardiogram (ECHO) or multigated acquisition (MUGA) scan
- Perform chest radiograph (may omit, if performed within 30 days prior to anticipated first dose)
- Record all concomitant medications including all prescription drugs, nonprescription drugs, and nutritional supplements within the past 2 weeks

5.2.2 Within 72 Hours Prior to First Dose (Stage 1, Stage 2)

Perform the following activities and evaluations within 72 hours prior to administration of the first dose of study drug:

- Collect blood for evaluation of laboratory parameters (*Appendix D*):
  - Hematology
  - Full serum chemistry panel
- Collect urine sample for full urinalysis
- Collect urine or serum sample for β-hCG pregnancy test (urine or serum) for females of child-bearing potential
- Record all concomitant medications including all prescription drugs, nonprescription drugs, and nutritional supplements

Review all Inclusion/Exclusion criteria and determine if patient has met all eligibility criteria for inclusion into the study.

Refer to the Study Manual of Procedures for detailed instructions on enrollment (and randomization for Stage 2).

5.3 Stage 1 Assessments

Sections 5.3.1 – 5.3.5.6 applies for all patients enrolled in Stage 1.

5.3.1 Assessments Required Prior to First Dose

Within 24 hours prior to first dose, patients will be hospitalized to receive supportive care measures and should remain hospitalized at least through Day 9. Patients will be discharged after Day 9 once deemed clinically stable by the investigator.

5.3.1.1 Cycle 1 – At Least 10 Hours Prior to First Dose

Perform the following procedures at least 10 hours prior to dosing on Day 1:

- Physical examination including weight (kg) for calculation of body surface area (BSA)
- Assess ECOG PS (*Appendix B*)
- Initiate supportive care measures at least 10 hours prior to first dose in all patients to minimize the likelihood of tumor lysis syndrome:
  - Administer pretreatment IV hydration, oral allopurinol, and oral phosphate binder (see *Section 4.6.1*)
  - Diligent monitoring of urine output **frequently** to ensure that it equals fluid input. If input is greater than output by 10%, administration of diuretics is encouraged. Replacement of excessive fluid losses, including from diarrhea, should be done unless otherwise clinically indicated.
- Record all concomitant medications including all prescription drugs, nonprescription drugs, and nutritional supplements taken since screening.

5.3.1.2 Cycle 1, Day 1 – Just Prior to First Dose

Perform the following procedures just prior to dosing on Day 1:

- Record vital signs (body temperature, heart rate, systolic and diastolic blood pressures) measured 5-15 minutes prior to the initiation of infusion following a 5-minute rest
- Collect blood for evaluation of laboratory parameters (*Appendix D*):
  - Tumor lysis labs to include electrolytes (sodium, potassium, chloride, and carbon dioxide) as well as creatinine, calcium, lactate dehydrogenase (LDH), uric acid, and phosphorus levels
  - Coagulation: fibrinogen level
- Collect urine or serum sample for β-hCG pregnancy test for females of child-bearing potential if screening pregnancy test is greater than 72 hours prior to first dose
- Record all concomitant medications including all nonprescription drugs and nutritional supplements

5.3.2 Cycle 1, Day 1 – Dosing

All Patients (regardless of risk of TLS):

- Monitor tumor lysis labs at the start of alvocidib and throughout the treatment cycle according to the schedule outlined in *Section 4.6.1.1*. [*Note:* there are different blood collection schedules for monitoring of TLS labs for patients at high risk of TLS and for those NOT at high risk for TLS.]
- Monitor fibrinogen levels as clinically indicated.
- Monitor urine output **frequently** to ensure that it equals fluid input. If input is greater than output by 10%, administration of diuretics is encouraged. Replacement of excessive fluid losses, including from diarrhea, should be done unless otherwise clinically indicated.
- Test for presence of *C. difficile* infection at first instance of diarrhea (refer to *Section 4.6.1.2* for additional guidance)
- Administer prophylactic antibiotics and antivirals, corticosteroid eye drops, and antifungals according to *Section 4.6.1.4*
- Assess for AEs
- Record only those medications (prescription, nonprescription, nutritional supplements) administered in conjunction with an AE.

5.3.2.1 Cycle 1 – Daily during Hospitalization for Chemotherapy

Perform the following procedures daily (or more frequently if clinically indicated) while patients are hospitalized for chemotherapy administration (ie, Days 2-9):

- Collect blood for evaluation of laboratory parameters (*Appendix D*):
  - Hematology
  - Tumor lysis labs as outlined in *Section 4.6.1.1* (or more frequently if clinically indicated)
  - Fibrinogen level (as clinically indicated)
- Monitor urine output **frequently** to ensure that it equals fluid input. If input is greater than output by 10%, administration of diuretics is encouraged. Replacement of excessive fluid losses, including from diarrhea, should be done unless otherwise clinically indicated.
- Test for presence of *C. difficile* infection at first instance of diarrhea (refer to *Section 4.6.1.2* for additional guidance)
- Assess for AEs
- Record only those medications (prescription, nonprescription, nutritional supplements) administered in conjunction with an AE.

5.3.3 Cycle 1 – Weekly after Completion of Chemotherapy Regimen

Perform the following procedures weekly (or more frequently if clinically indicated) after patients have completed their chemotherapy regimen:

- Abbreviated physical examination (AE- or symptom-directed exam) including weight (kg)
- Vital signs (temperature, heart rate, systolic and diastolic blood pressures)
- Collect blood for evaluation of laboratory parameters (*Appendix D*):
  - Hematology
  - Tumor lysis labs
  - Fibrinogen level (as clinically indicated)
- Assess ECOG PS (*Appendix B*)
- Assess for AEs
- Record only those medications (prescription, nonprescription, nutritional supplements) administered in conjunction with an AE.

5.3.4 Cycle 1 – At Hematologic Recovery

- Assess response by bone marrow biopsy and/or aspiration at time of hematologic recovery (ie, absolute neutrophil count (ANC) >1000 µL and platelet count >100,000 µL) or Day 45 (±2), whichever occurs first. If the bone marrow biopsy and/or aspirate is nonproductive or not diagnostic, the procedure must be repeated within 7 days.
  - At time of response assessment, 6 to 8 bone marrow slides will be prepared and sent to the Sponsor. Details regarding the collection and shipping of these slides is provided in the Study Manual.
- Assess for AEs, including tumor lysis clinical symptoms
- Record only those medications (prescription, nonprescription, nutritional supplements) administered in conjunction with an AE.

5.3.5 Cycles 2 - 4

Patients who achieve CR, CRi, CRp, or PR after the first cycle (completion of all doses) may receive up to 3 additional optional cycles of treatment. After completing the first cycle of treatment, continued use of mitoxantrone is optional. Mitoxantrone must be omitted from subsequent cycles if the patient's lifetime daunorubicin equivalent exceeds 460 mg/m^2 (see *Appendix G* for conversion table) or the LVEF drops below 45%.

Patients not demonstrating evidence of CR, CRi, CRp, or PR after the first cycle of treatment will be considered for removal from the study, although with permission of the Medical Monitor, induction treatment may continue if clinically indicated and provided there is no evidence of toxicity ≥NCI CTCAE grade 4 (v4.03 in Stage 1, v5.0 in Stage 2; see *Appendix C*).

There is a maximum limit of 90 days between the start of cycles unless otherwise discussed with the medical monitor.

5.3.5.1 Cycles 2 - 4, Day 1 – At Least 10 Hours Prior to First Dose

Perform the following assessments in patients receiving subsequent cycles of chemotherapy:

- If clinically indicated, administer pretreatment IV hydration, oral allopurinol, and oral phosphate binder (see *Section 4.6.1*)
- If supportive care measures for prevention of tumor lysis are initiated, monitor urine output **frequently** to ensure that it equals fluid input. If input is greater than output by 10%, administration of diuretics is encouraged. Replacement of excessive fluid losses, including from diarrhea, should be done unless otherwise clinically indicated.
- ECHO or MUGA scan to be performed before each dose of mitoxantrone. If mitoxantrone is omitted after Cycle 1, the ECHO or MUGA scan can also be omitted.

5.3.5.2 Cycles 2 - 4, Day 1 – Just Prior to First Dose

Perform the following procedures just prior to dosing on Day 1:

- Physical examination including weight (kg) for recalculation of BSA
- Record vital signs measured 5-15 minutes prior to the initiation of infusion following a 5-minute rest
- Assess ECOG PS (*Appendix B*)
- Collect blood for evaluation of laboratory parameters (*Appendix D*):
    - Hematology
    - Tumor lysis labs
    - Fibrinogen level (as clinically indicated)
- If supportive care measures for prevention of tumor lysis were initiated, monitor urine output **frequently** to ensure that it equals fluid input. If input is greater than output by 10%, administration of diuretics is encouraged. Replacement of excessive fluid losses, including from diarrhea, should be done unless clinically indicated.
- Collect urine or serum sample for β-hCG pregnancy test for females of child-bearing potential.

- Record only those medications (prescription, nonprescription, nutritional supplements) administered in conjunction with an AE.

5.3.5.3 Cycles 2 - 4, Day 1 – Dosing

- Collect blood for evaluation of laboratory parameters (*Appendix D*):
  - Hematology
  - Tumor lysis labs
  - Fibrinogen level (as clinically indicated)
- If supportive care measures for prevention of tumor lysis were initiated, monitor urine output **frequently** to ensure that it equals fluid input. If input is greater than output by 10%, administration of diuretics is encouraged. Replacement of excessive fluid losses, including from diarrhea, should be done unless otherwise clinically indicated.
- Test for presence of *C. difficile* infection at first instance of diarrhea (refer to *Section 4.6.1.2* for additional guidance)
- Administer prophylactic antibiotics and antivirals, corticosteroid eye drops, and antifungals according to *Section 4.6.1.4*.
- Assess for AEs
- Record only those medications (prescription, nonprescription, nutritional supplements) administered in conjunction with an AE.

5.3.5.4 Cycles 2 - 4 – Daily during Hospitalization for Chemotherapy

Perform the following procedures daily (or more frequently if clinically indicated) while patients are hospitalized for chemotherapy administration (ie, Days 2-9):

- Collect blood for evaluation of laboratory parameters (*Appendix D*):
  - Hematology
  - Tumor lysis labs
  - Fibrinogen level (only required if clinically indicated)
- If supportive care measures to prevent/treat tumor lysis were initiated, must monitor urine output **frequently** to ensure that it equals fluid input. If input is greater than output by 10%, administration of diuretics is encouraged. Replacement of excessive fluid losses, including from diarrhea, should be done unless otherwise clinically indicated.
- Test for presence of *C. difficile* infection at first instance of diarrhea (refer to *Section 4.6.1.2* for additional guidance)
- Assess for AEs
- Record only those medications (prescription, nonprescription, nutritional supplements) administered in conjunction with an AE.

5.3.5.5 Cycles 2 - 4 – Weekly after Completion of Chemotherapy Regimen

Perform the following procedures weekly (or more frequently if clinically indicated) after patients have completed their chemotherapy regimen:

- Abbreviated physical examination including weight (kg)
- Vital signs (temperature, heart rate, systolic and diastolic blood pressures)
- Assess ECOG PS (*Appendix B*)
- Collect blood for evaluation of laboratory parameters (*Appendix D*):
    - Hematology
    - Tumor lysis labs
    - Fibrinogen level (only required if clinically indicated)
- Assess for AEs
- Record only those medications (prescription, nonprescription, nutritional supplements) administered in conjunction with an AE.

5.3.5.6 Cycles 2 - 4 – At Hematologic Recovery

- Assess response by bone marrow biopsy and/or aspiration at time of hematologic recovery (ie, absolute neutrophil count (ANC) >1000 µL and platelet count >100,000 µL) or Day 45 (±2), whichever occurs first. If the bone marrow biopsy and/or aspirate is nonproductive or not diagnostic, the procedure must be repeated within 7 days.
    - At time of response assessment, 6 to 8 bone marrow slides will be prepared and sent to the Sponsor. Details regarding the collection and shipping of these slides is provided in the Study Manual.
- Assess for AEs, including tumor lysis clinical symptoms
- Record only those medications (prescription, nonprescription, nutritional supplements) administered in conjunction with an AE.

5.4 Stage 2 Assessments

Sections 5.4.1 – 5.4.5.6 applies for all patients enrolled in Stage 2.

5.4.1 Assessments Required Prior to First Dose

5.4.1.1 Cycle 1, Day 1 – At Least 10 Hours Prior to First Dose

Perform the following procedures at least 10 hours prior to dosing on Day 1:

- Physical examination including weight (kg) for calculation of body surface area (BSA)
- Assess ECOG PS (*Appendix B*)
- Initiate supportive care measures at least 10 hours prior to first dose in all patients to minimize the likelihood of tumor lysis syndrome:
    - Administer pretreatment IV hydration, oral allopurinol, and oral phosphate binder (see *Section 4.6.1*)
    - Diligent monitoring of urine output **frequently** to ensure that it equals fluid input. If input is greater than output by 10%, administration of diuretics is encouraged. Replacement of excessive fluid losses, including from diarrhea, should be done unless otherwise clinically indicated.
- Record all concomitant medications including all prescription drugs, nonprescription drugs, and nutritional supplements taken since screening.

5.4.1.2 Cycle 1, Day 1 – Just Prior to First Dose

Perform the following procedures just prior to dosing on Day 1:

- Record vital signs (body temperature, heart rate, systolic and diastolic blood pressures) measured 5-15 minutes prior to the initiation of infusion following a 5-minute rest
- Collect blood for evaluation of laboratory parameters (*Appendix D*):
    - Tumor lysis labs to include electrolytes (sodium, potassium, chloride, and carbon dioxide) as well as creatinine, calcium, lactate dehydrogenase (LDH), uric acid, and phosphorus levels.
    - Coagulation: fibrinogen level
- Collect urine or serum sample for β-hCG pregnancy test for females of child-bearing potential if screening pregnancy test is greater than 72 hours prior to first dose.
- Record all concomitant medications including all nonprescription drugs and nutritional supplements.

5.4.2 Cycle 1, Day 1 – Dosing

All Patients (regardless of risk of TLS):

- Monitor tumor lysis labs at the start of treatment (alvocidib for ACM, cytarabine for CM) and throughout the treatment cycle according to the schedule outlined in *Section 4.6.1.1*. [*Note*: there are different blood collection schedules for monitoring of TLS labs for patients at high risk of TLS and for those NOT at high risk for TLS.]
- Monitor fibrinogen levels as clinically indicated.
- Monitor urine output **frequently** to ensure that it equals fluid input. If input is greater than output by 10%, administration of diuretics is encouraged. Replacement of excessive fluid losses, including from diarrhea, should be done unless otherwise clinically indicated.
- Test for presence of *C. difficile* infection at first instance of diarrhea (refer to *Section 4.6.1.2* for additional guidance)
- Administer prophylactic antibiotics and antivirals, corticosteroid eye drops, and antifungals according to *Section 4.6.1.4*.
- Assess for AEs
- Record only those medications (prescription, nonprescription, nutritional supplements) administered in conjunction with an AE.

5.4.2.1 Cycle 1 – Daily during Hospitalization for Chemotherapy

Perform the following procedures daily (or more frequently if clinically indicated) while patients are hospitalized for chemotherapy administration (ie, Days 2-9 for patients receiving **ACM**: Days 1-3 for patients receiving **CM**):

- Collect blood for evaluation of laboratory parameters (*Appendix D*):
  - Hematology
  - Tumor lysis labs daily (or more frequently if clinically indicated)
  - Fibrinogen level (only required if clinically indicated)
- Monitor urine output **frequently** to ensure that it equals fluid input. If input is greater than output by 10%, administration of diuretics is encouraged. Replacement of excessive fluid losses, including from diarrhea, should be done unless otherwise clinically indicated.
- Test for presence of *C. difficile* infection at first instance of diarrhea (refer to *Section 4.6.1.2* for additional guidance)
- Assess for AEs
- Record only those medications (prescription, nonprescription, nutritional supplements) administered in conjunction with an AE.

5.4.3 Cycle 1 – Weekly after Completion of Chemotherapy Regimen

Perform the following procedures weekly (or more frequently if clinically indicated) after patients have completed their chemotherapy regimen:

- Abbreviated physical examination including weight (kg)
- Vital signs (temperature, heart rate, systolic and diastolic blood pressures)
- Collect blood for evaluation of laboratory parameters (*Appendix D*):
    - Hematology
    - Tumor lysis labs
    - Fibrinogen level (only required if clinically indicated)
- Assess for AEs
- Record only those medications (prescription, nonprescription, nutritional supplements) administered in conjunction with an AE.

5.4.4 Cycle 1 – At Hematologic Recovery

- Assess response by bone marrow biopsy and/or aspiration at time of hematologic recovery (ie, absolute neutrophil count (ANC) >1000 µL and platelet count >100,000 µL) or Day 45 (±2), whichever occurs first. If the bone marrow biopsy and/or aspirate is nonproductive or not diagnostic, the procedure must be repeated within 7 days.
    - At time of response assessment, 6 to 8 bone marrow slides will be prepared and sent to the Sponsor. Details regarding the collection and shipping of these slides is provided in the Study Manual.
- Assess for AEs, including tumor lysis clinical symptoms
- Record only those medications (prescription, nonprescription, nutritional supplements) administered in conjunction with an AE.

5.4.5 Cycles 2 - 4

Patients who achieve CR, CRi, CRp, or PR after the first cycle (completion of all doses) may receive up to 3 additional optional cycles of treatment. After completing the first cycle of treatment, continued use of mitoxantrone is optional. Mitoxantrone must be omitted from subsequent cycles if the patient's lifetime daunorubicin equivalent exceeds 460 mg/m^2 (see *Appendix G* for conversion table) or the LVEF drops below 45%.

Patients not demonstrating evidence of CR, CRi, CRp, or PR after the first cycle of treatment will be considered for removal from the study, although with permission of the Medical Monitor, induction treatment may continue if clinically indicated and provided there is no evidence of toxicity ≥NCI CTCAE grade 4.

However, patients randomized to CM with progressive disease or no response after 1 cycle of CM may cross over to receive ACM. In addition, patients randomized to CM with the best response of a PR after 2 cycles of CM may also cross over to receive ACM. Patients who cross over to ACM may receive up to a combined total (CM + ACM) of 4 cycles.

There is a maximum limit of 90 days between the start of cycles unless otherwise discussed with the medical monitor.

5.4.5.1 Cycles 2 - 4, Day 1 – Within 10 Hours Prior to First Dose

Perform the following assessments in patients receiving subsequent cycles of chemotherapy:

- *If clinically indicated*, administer pretreatment IV hydration, oral allopurinol, and oral phosphate binder (see *Section 4.6.1*)
- If supportive care measures for prevention of tumor lysis are initiated, monitor urine output **frequently** to ensure that it equals fluid input. If input is greater than output by 10%, administration of diuretics is encouraged. Replacement of excessive fluid losses, including from diarrhea, should be done unless otherwise clinically indicated.
- ECHO or MUGA scan to be performed before each dose of mitoxantrone. If mitoxantrone is omitted after Cycle 1, the ECHO or MUGA scan can also be omitted.

5.4.5.2 Cycles 2 - 4, Day 1 – Just Prior to First Dose

Perform the following procedures just prior to dosing on Day 1:

- Physical examination including weight (kg) for recalculation of BSA
- Record vital signs measured 5-15 minutes prior to the initiation of infusion following a 5-minute rest
- Assess ECOG PS (*Appendix B*)
- Collect blood for evaluation of laboratory parameters (*Appendix D*):
    - Hematology
    - Tumor lysis labs
    - Fibrinogen level (only required if clinically indicated)
- If supportive care measures for prevention of tumor lysis were initiated, monitor urine output **frequently** to ensure that it equals fluid input. If input is greater than output by 10%, administration of diuretics is encouraged. Replacement of excessive fluid losses, including from diarrhea, should be done unless otherwise clinically indicated.

- Collect urine or serum sample for β-hCG pregnancy test for females of child-bearing potential
- Record only those medications (prescription, nonprescription, nutritional supplements) administered in conjunction with an AE.

5.4.5.3 Cycles 2 - 4, Day 1 – Dosing

- Treatment with **ACM** or **CM**.

  After completing the first cycle of treatment, continued use of mitoxantrone is optional.
- Collect blood for evaluation of laboratory parameters (*Appendix D*):
  - Hematology
  - Tumor lysis labs
  - Fibrinogen level (only required if clinically indicated)
- If supportive care measures for prevention of tumor lysis were initiated, monitor urine output **frequently** to ensure that it equals fluid input. If input is greater than output by 10%, administration of diuretics is encouraged. Replacement of excessive fluid losses, including from diarrhea, should be done unless otherwise clinically indicated.
- Test for presence of *C. difficile* infection at first instance of diarrhea (refer to *Section 4.6.1.2* for additional guidance)
- Administer prophylactic antibiotics and antivirals, corticosteroid eye drops, and antifungals according to *Section 4.6.1.4*.
- Assess for AEs
- Record only those medications (prescription, nonprescription, nutritional supplements) administered in conjunction with an AE.

5.4.5.4 Cycles 2 - 4 – Daily during Hospitalization for Chemotherapy

Perform the following procedures daily (or more frequently if clinically indicated) while patients are hospitalized for chemotherapy administration:

- Collect blood for evaluation of laboratory parameters (*Appendix D*):
  - Hematology
  - Tumor lysis labs
  - Fibrinogen level (only required if clinically indicated)

- If supportive care measures to prevent/treat tumor lysis were initiated, must monitor urine output **frequently** to ensure that it equals fluid input. If input is greater than output by 10%, administration of diuretics is encouraged. Replacement of excessive fluid losses, including from diarrhea, should be done unless otherwise clinically indicated.
- Test for presence of *C. difficile* infection at first instance of diarrhea (refer to *Section 4.6.1.2* for additional guidance)
- Assess for AEs
- Record only those medications (prescription, nonprescription, nutritional supplements) administered in conjunction with an AE.

5.4.5.5 Cycles 2 - 4 – Weekly after Completion of Chemotherapy Regimen

Perform the following procedures weekly (or more frequently if clinically indicated) after patients have completed their chemotherapy regimen:

- Abbreviated physical examination including weight (kg)
- Vital signs (temperature, heart rate, systolic and diastolic blood pressures)
- Assess ECOG PS (*Appendix B*)
- Collect blood for evaluation of laboratory parameters (*Appendix D*):
  - Hematology
  - Tumor lysis labs
  - Fibrinogen level (only required if clinically indicated)
- Assess for AEs
- Record only those medications (prescription, nonprescription, nutritional supplements) administered in conjunction with an AE.

5.4.5.6 Cycles 2 - 4 – At Hematologic Recovery

- Assess response by bone marrow biopsy and/or aspiration at time of hematologic recovery (ie, absolute neutrophil count (ANC) >1000 µL and platelet count >100,000 µL) or Day 45 (±2), whichever occurs first. If the bone marrow biopsy and/or aspirate is nonproductive or not diagnostic, the procedure must be repeated within 7 days.
  - At time of response assessment, 6 to 8 bone marrow slides will be prepared and sent to the Sponsor. Details regarding the collection and shipping of these slides is provided in the Study Manual.
- Assess for AEs, including tumor lysis clinical symptoms

- Record only those medications (prescription, nonprescription, nutritional supplements) administered in conjunction with an AE.

5.5 End of Study Assessments (Stage 1, Stage 2)

To be completed at time patient is withdrawn from the study.

- Abbreviated physical examination including weight (kg) and other measures of disease and disease symptoms, eg, extramedullary disease
- Vital signs (temperature, heart rate, systolic and diastolic blood pressures)
- ECOG PS (*Appendix B*)
- Collect blood for evaluation of laboratory studies (*Appendix D*):
    - Hematology
    - Full serum chemistry panel
    - Fibrinogen level (only required if clinically indicated)
- Urinalysis
- Perform bone marrow biopsy and/or aspiration (if not done in the preceding 30 days). If the bone marrow biopsy and/or aspirate is nonproductive or not diagnostic, the procedure must be repeated.
    - At end of study, 6 to 8 bone marrow slides will be prepared and sent to the Sponsor (if not done in the preceding 30 days). Details regarding the collection and shipping of these slides is provided in the Study Manual.
- Perform 12-lead ECG (as clinically indicated)
- Perform ECHO or MUGA scan (as clinically indicated)
- Assess for AEs
- Record only those medications (prescription, nonprescription, nutritional supplements) administered in conjunction with an AE

5.6 Follow-up Assessments (Stage 1, Stage 2 and Exploratory Arms)

All study patients will be contacted by telephone to assess for date of death, date of stem cell transplant, date of relapse, or continued remission for two years:

- Year 1: Phone calls monthly beginning the month after the patient completes the end-of-study assessments to 12 months after Day of Randomization regardless of how many cycles a patient receives (Year 1 = Day of Randomization plus 12 months)
- Year 2: Phone calls every other month during Year 2 (months 14 to 24 after Day of Randomization).

6. OFF-STUDY CRITERIA

6.1 Withdrawal of Patients

All patients have the right to withdraw at any time during treatment without prejudice. Circumstances may occur under which a patient may be permanently removed from the study. The criteria used to justify withdrawal of a study patient are described below.

In the event of a premature withdrawal, the assessments for the End of Study visit, as detailed in the Schedule of Activities (See *Appendix A*), should be completed at the time of the withdrawal, wherever possible, including dates of remission and death. If the study patient is prematurely withdrawn due to an adverse event(s), attempts should also be made to clinically follow the study patient until the event is resolved, stable or permanent as determined by the PI and Sponsor.

6.2 Reasons for Withdrawal

A patient may be permanently removed from the study for any of the following reasons:

- Failure to achieve a CR or PR. Patients not demonstrating evidence of CR, CRi, CRp, or PR after the first cycle of treatment will be considered for removal from the study, although with permission of the Medical Monitor, induction treatment may continue if clinically indicated and provided there is no evidence of toxicity ≥NCI CTCAE grade 4 (v4.03 in Stage 1, v5.0 in Stage 2). See *Section 4.5.3* for criteria for patients randomized to CM that may cross over to ACM.
- An excessive grade 3-4 toxicity without a response to treatment or occurrence of any other adverse event, concurrent illness or laboratory abnormality which, in the opinion of the PI, warrants the patient's permanent withdrawal
- Patient noncompliance, defined as refusal or inability to adhere to the study schedule;
- At the request of the patient, PI, the Sponsor, or regulatory authority;
- Patient is lost to follow-up; or
- Patient death
- Patient becomes pregnant while on study

6.3 Follow-up for Patients Withdrawn from Study

Patients withdrawn from the study with an ongoing adverse event must be followed clinically until the event is resolved, deemed stable or permanent by the PI, or the patient begins a new treatment for their disease. A stable adverse event is defined as an event that is not expected to change in nature, severity or frequency. See *Sections 8.4 – 8.8* for reporting of adverse events. Patients withdrawn from the study for pregnancy will be followed according to *Section 5.6* Follow-up Assessments. The pregnancy of any patient, or patient's partner, will be followed to term to record any birth defects/abnormalities at time of birth.

7. CRITERIA FOR EVALUATION AND ENDPOINT DEFINITIONS

7.1 Efficacy Endpoints

Response assessments in Stage 1 are defined by the International Working Group Criteria and 2010 European LeukemiaNet (ELN) [20, 21]. The 2017 ELN criteria will be used to determine patient responses during Stage 2 [23]. These criteria are summarized in *Section 11.4.2* and provided in *Appendix E*.

7.1.1 Primary Efficacy Endpoint

- CR rate

7.1.2 Secondary Efficacy Endpoints

- Combined CR rate
- Combined Response Rate
- Overall Survival (OS)
- Event-free Survival (EFS)
- Rate of Stem Cell Transplantation

7.2 Safety Endpoints

Tolerance and toxicity of the ACM versus CM regimens will be assessed through evaluation of physical examinations, vital signs, laboratory studies, solicited and unsolicited adverse events, and all causes of mortality at 30 and 60 days.

- 30- and 60-day Mortality
- Adverse Events
- Clinical Laboratory Assessments

A Data Safety Monitoring Board (DSMB) will monitor key outcomes from the study.

8. ADVERSE EVENTS

8.1 Definitions

An adverse event (AE) is defined as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, whether or not related to the drug product.)

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event.

Unexpected adverse event or unexpected suspected adverse reaction: An adverse event or suspected adverse reaction is considered unexpected if it is not listed in the current Investigator's Brochure or is not listed at the specificity or severity that has been observed; or, is not consistent with the risk information described in the general investigational plan (clinical study protocol).

Toxicities will be assessed according to the NCI CTCAE, version 4.03 during Stage 1 and v5.0 during Stage 2 (see *Appendix C*). When the NCI CTCAE grade is not available, the investigator will use the following toxicity grading: mild, moderate, severe, life-threatening or fatal.

SEVERITY	***CTCAE v4.03 (Stage 1)***	***CTCAE v5.0 (Stage 2)***
GRADE 1 – Mild:	Transient or mild discomfort; no limitation in activity; no medical intervention/therapy required.	Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
GRADE 2 – Moderate:	Mild to moderate limitation in activity - some assistance may be needed; no or minimal medical intervention/therapy required.	Minimal, local or noninvasive intervention indicated; limiting age-appropriate instrumental ADL [a]
GRADE 3 – Severe:	Marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalizations possible.	Medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL [b]
GRADE 4 – Life Threatening:	Extreme limitation in activity, significant assistance required; life threatening (immediate risk of death); significant medical intervention/therapy required, hospitalization or hospice care probable.	Life-threatening consequences; urgent intervention indicated
GRADE 5 – Fatal:	Results in death.	Death related to AE

a Instrumental activities of daily living (ADLs) refer to preparing meals, shopping for groceries or clothes, using the telephone, managing money, etc.
b Self-care ADLs refer to bathing, dressing and undressing, feeding self, using the toilet, taking medications, and not bedridden.

8.2 Causality

Relationship of the adverse event (AE) to the study drug should be defined as follows:

Unrelated:	AE is *clearly not related* to the investigational agent(s)
Unlikely:	AE is *doubtfully related* to the investigational agent(s)
Possible:	AE *may be related* to the investigational agent(s)
Probable:	AE is *likely related* to the investigational agent(s)
Definite:	AE is *clearly related* to the investigational agent(s)

8.3 Serious Adverse Events

A serious adverse event (SAE) is defined as any experience that suggests a significant hazard, contraindication, side effect, or precaution. An SAE includes:

- Any death, or
- Any life-threatening event (ie, the patient is at immediate risk of death from the event as it occurred), or
- Any event that is persistently, significantly, severely or permanently disabling, or requires intervention to prevent such disability, or
- Any event which requires inpatient hospitalization or prolongs hospitalization, or
- Any congenital abnormality/birth defect, or
- Any medically significant event that may jeopardize the patient or may require intervention to prevent one of the other outcomes listed above.

In addition, any adverse event which results in termination of the patient from study will be considered a potentially serious adverse event and must be reported to the Sponsor as described in *Section 8.4* below.

Bone marrow suppression and associated complications are expected events during leukemia therapy and are part of the treatment process (marrow emptying of leukemic cells). Therefore, myelosuppression and associated complications directly related to the myelosuppression, such as fever, infections and bleeding, will not be reported as serious adverse events (SAEs), but will be reported as adverse events on the adverse event CRF and will be summarized in the updated and final reports. Anemia and thrombocytopenia will not be reported as an SAE. Prolonged bone marrow suppression (as defined by the NCI toxicity criteria specific for leukemia, ie, bone marrow cellularity <5% on day 42 or later (6 weeks) from start of therapy without evidence of leukemia) or the unexpected nature, severity or frequency of myelosuppression, anemia, and thrombocytopenia or an associated complication will be reported as an SAE.

8.4 Eliciting and Reporting Adverse Events

All adverse events, regardless of severity, which occur during the study, will be documented in the study progress notes, and the “AE” CRF will be completed. This includes both serious and non-serious events. Adverse events occurring from the time of the first dose will be captured.

All adverse events noted by study staff or volunteered by study patients at any time will be recorded. Any unexpected AE ≥NCI CTCAE Grade 3 that occurs during this study and up to 30 days after discontinuation of study drug (alvocidib in Stage 1; alvocidib, cytarabine or mitoxantrone in Stage 2) or alternate cancer therapy, whichever occurs first, regardless of relationship, should be reported to the Medical Monitor at the next investigator call. The Investigator or a qualified designated staff physician will conduct clinical assessments of all patients at each scheduled clinic visit. In addition, patients will be queried about any adverse symptoms they have experienced since the previous study visit. In order to avoid bias in eliciting events, suggestive questioning of the patients shall not occur.

For Stage 1 (CTCAE v4.03) and Stage 2 (CTCAE v5.0) patients, record a laboratory abnormality as an AE if it is associated with clinical sequelae or requires a therapeutic intervention. For both non-laboratory and laboratory abnormalities capture only the highest grade of an event using Start/Stop dates of the longest duration of the AE not the longest duration of the highest grade. If worst grade is captured on a lab drawn between visits, enter the lab under Unscheduled Evaluations. Additional details are provided in the electronic case report form (eCRF) Completion Guidelines.

Adverse events will be reported and described in terms of intensity, seriousness and causality, based on the Investigator’s judgment using protocol-defined definitions. Necessary counter measures will also be reported on the appropriate CRF used to collect concomitant medications.

8.5 Serious Adverse Events and/or Adverse Events Requiring Discontinuation of Study Drug

Any serious adverse event (SAE) that occurs during this study and up to 30 days after discontinuation of study drug (alvocidib in Stage 1; alvocidib, cytarabine or mitoxantrone in Stage 2) or initiation of alternate cancer therapy, whichever occurs first, must be reported to the Study Medical Monitor within 24 hours of the Investigator's awareness of the event, whether or not this reaction is considered to be associated with use of the investigational drug.

In addition, the occurrence of any AE leading to permanent discontinuation of study drug must also be reported to the Sponsor within 24 hours of the Investigator's awareness of the event.

Serious adverse events must be scanned and emailed to the Sponsor/Study Medical Monitor.

Email: [redacted]

It is expected that the Investigator will provide or arrange appropriate supportive care for the study patient. A patient experiencing an SAE(s) should be followed clinically until the event is resolved, deemed stable or permanent by the PI, or the patient begins a new treatment for their disease. All telephone and scanned/emailed reports must be followed with a written SAE report form within 24 hours of the Investigator's awareness of serious adverse events and nonserious events which required discontinuation of study drug.

The SAE report form should be completed and signed by the Investigator, scanned, and sent by email to the Sponsor as described above. The SAE Report Form is distinct and separate from the adverse event form included in the CRF.

Grades for all SAEs and AEs, regardless of whether they trigger expedited reporting or not, must still be captured by the CRF.

8.6 Follow-up of Adverse Events

Adverse events, which are identified on the last scheduled visit, must be recorded on the AE CRF page and reported to the Sponsor according to the procedures outlined in *Section 8.4*.

Patients with unresolved previously reported adverse events or new adverse events identified on the last scheduled visit should be followed by the Investigator until the events resolve, are deemed stable or permanent, or the patient begins another treatment for their disease. Resolution means the patient has returned to his/her baseline state of health or the Investigator does not expect any further improvement or worsening of the adverse event. The Investigator should continue to report any significant follow-up information to the Sponsor up to the point the event has resolved. Any adverse events reported by the patient to the Investigator which occur after the last scheduled visit, and are determined by the Investigator to be reasonably associated with the use of the

study drug or meet the criteria of a reportable adverse event as described above, should be reported to the Sponsor.

Patients withdrawn from the study with an ongoing adverse event must be followed clinically until the event is resolved, deemed stable or permanent as determined by the Investigator and Sponsor, or the patient begins another treatment for their disease. A stable adverse event is defined as an event, which is not expected to change in nature, severity, or frequency. The Investigator should continue to report any significant follow-up information to the Sponsor.

8.7 Patient Deaths

Every effort will be made in the case of patients who die to determine the cause of death. Information regarding a patient who dies more than 30 days after receiving study drug may be recorded on a Death Report Form (no SAE report is required). An SAE report is recorded only if the death occurs within 30 days of the last administration of study drug (alvocidib in Stage 1; alvocidib, cytarabine or mitoxantrone in Stage 2).

The Death Report Form is distinct and separate from the adverse event form included in the CRF.

8.8 Reporting Adverse Events to the Regulatory Authorities

The Sponsor will be responsible for reporting adverse events to the Food and Drug Administration (FDA) as described in 21 Code of Federal Regulations (CFR) Section 312.32 (IND Safety Reports) and to other Regulatory Authorities according to local regulations.

In addition, the Investigator is required by FDA regulations to notify the Institutional Review Board (IRB) promptly of all unexpected SAEs occurring at the investigator's study site. The Investigator is also required by FDA regulations to forward the IRB all IND Safety Reports received from the sponsor.

The Sponsor will also report SAEs in compliance with local regulatory requirements.

9. STUDY DRUG MANAGEMENT

9.1 Study Drug

The investigational study drug, alvocidib, will be provided to the PI by the Sponsor or designee.

Alvocidib is supplied for parenteral administration as a sterile, nonpyrogenic, injectable, clear pale yellow to yellow-colored, 10 mg/mL solution, which is packaged in glass vials fitted with coated rubber closures crimped with an aluminum seal and light blue plastic cap. Each vial contains 50 mg of alvocidib (calculated with reference to the active moiety). The fill volume has been established to ensure removal of 5 mL. The pH of the solution ranges between 2.7 and 3.3. The solution contains the following excipients: water for injection, glacial acetic acid, and sodium hydroxide (as needed to reach the targeted pH).

Alvocidib is to be diluted with either 0.9% sodium chloride injection or 5% dextrose injection prior to infusion, providing solutions of 0.09 to 1.0 mg/mL alvocidib.

Cytarabine and mitoxantrone, approved pharmaceutical products, will be provided by commercially available sources.

9.2 Study Drug Dispensing and Accountability

Alvocidib will be provided by the Sponsor to study centers as an investigational drug. The label and package for the drug product will be prepared in accordance with current regulatory requirements. The Investigator or designee will inventory and acknowledge receipt of all shipments of study drugs. The study drugs must be kept in a locked area with access restricted to designated study personnel.

An accurate and current accounting of the dispensing of the study drugs for each patient will be maintained on an ongoing basis by a member of the study site staff in a drug accountability log or equivalent document and will be verified by the sponsor's study monitor. All drug supplies, including unused study drug, must be accounted for. A final inventory of the total amount of drug received at each study site against the amount used and returned must be recorded in the study drug accountability log or an equivalent document. Inventory and dispense records must be readily available for inspection by the study monitor and/or auditor, and open to government inspection at any time. Study drug destruction will be handled by the sites of open/used vials. Unopened study drug vials should be returned to the Sponsor or the Contract Research Organization (ie, Courante) at the end of the study only after full drug accountability has been completed by the study monitor.

9.3 Preparation and Administration

Alvocidib is to be diluted with either 0.9% Sodium Chloride Injection or 5% Dextrose Injection prior to infusion, providing solutions of 0.09 to 1.0 mg/mL alvocidib. The diluted solution should be administered according to treatment schedule provided in *Section 4.5*.

Cytarabine (Ara-c), using the 2-gm vial, may be reconstituted with 20 mL of Bacteriostatic Water for Injection with Benzyl Alcohol 0.945% w/v added as preservative. The resulting solution contains 100 mg of cytarabine per mL. Administer according to treatment schedule in *Section 4.5*.

Mitoxantrone is supplied as a concentrate that MUST BE DILUTED PRIOR TO INJECTION. The dose of mitoxantrone should be diluted to at least 50 mL with either 0.9% Sodium Chloride Injection or 5% Dextrose Injection. Mitoxantrone may be further diluted into Dextrose 5% in Water, Normal Saline or Dextrose 5% with Normal Saline and used immediately. Mitoxantrone should not be mixed in the same infusion as heparin since a precipitate may form. Because specific compatibility data are not available, it is recommended that mitoxantrone not be mixed in the same infusion with other drugs. Administer according to treatment schedule in *Section 4.5*.

Both cytarabine and mitoxantrone are approved pharmaceutical products. Complete instructions and training on the proper preparation and administration of all study drugs will be provided to study sites in the Pharmacy Manual.

9.4 Storage at Study Center

- Alvocidib should be stored between 2° to 30°C (36° to 86°F).
- Cytarabine (Ara-c) should be stored between 15° to 30°C (59° to 86°F).
- Mitoxantrone (mitoxantrone hydrochloride) should be stored between 20° to 25°C (68° to 77°F). DO NOT FREEZE.

9.5 Compliance

Study drugs will be administered by trained staff at the treatment site(s).

10. RECORD MANAGEMENT

10.1 Data Collection

The Investigator must maintain required records for all study subjects. Case report forms are used to record clinical study data and are an integral part of the study and subsequent reports. Data for this study will be recorded in the subject's source document and into an eCRF system that must be kept current to reflect patient status during each part of the study. Patients are not to be identified by name on the eCRF. Appropriately coded identification (site number, patient identification number, and patient initials) should be used.

Electronic CRFs are not to be used as source documents. Investigators must keep accurate separate records (other than the CRFs) of all subjects' visits, being sure to include all pertinent study related information. A statement should be made indicating that the subjects have been enrolled in this clinical study and have provided written Informed Consent. Any adverse events must be thoroughly documented. Results of any diagnostic tests conducted during the study should also be included in the source documentation.

All data should be recorded completely and promptly in the eCRFs as soon after the visit as possible, but no later than 5 days. All queries are to be answered within 3 days of query date.

The Principal Investigator will allow the Sponsor or its representative, or an appropriate representative of the regulatory authorities to inspect study documents (eg, consent forms, drug distribution forms, IRB approval) and pertinent hospital or clinic records for confirmation of data throughout the study period.

10.2 Source Document Maintenance

Source documents are defined as the results of original observations and activities of a clinical investigation. Source documents may include, but are not limited to, hospital medical records, study progress notes, consent forms, computer printouts, laboratory data and recorded data from automated instruments. All source documents produced in this study will be maintained by the PI and made available for inspection by representatives of the Sponsor or the Regulatory Authorities. The original signed informed consent form for each participating patient shall be filed with the records kept by the PI with a copy filed in the patient’s medical records, and a copy given to the patient.

A source document is an original record of information, also known as source data, which is necessary for the reconstruction and evaluation of a clinical trial. The purpose of source documents is to provide proof of a participant’s existence, confirm that protocol-related procedures were completed and conducted per protocol and to verify that data reported in the study CRFs are accurate.

Source documents at a clinical trial site may be maintained in paper or electronic format and typically contain the types of information below. If electronic source documents are used, sponsor and study monitors will be given access to verify study data.

Source documents can include, but are not limited to:

- Notes from clinic physicians, nurses, and other study staff
- Reports of procedures and tests
- Flow sheets, checklists, and worksheets
- Subject diaries, study calendars
- Pharmacy records, accountability logs, shipping receipts
- Study notes or memos to file
- Documented telephone calls, emails, faxes
- Hospital admission forms and discharge summaries
- Sponsor/site-generated study source document templates

Source documents must meet five fundamental principles of data quality ("ALCOA"). They must be:

- **A**ttributable – The data originator is identified. If data needs to be amended, the amender is identified.
- **L**egible – The source document must be readable. If handwritten, black or blue ink must be used, never pencil.
- **C**ontemporaneous – The document must be signed and dated when the information is first recorded, with any updates or corrections noted in real time as well.
- **O**riginal – The document must be the first place the information is recorded.
- **A**ccurate – The information must be error-free, and any conflicts with data recorded elsewhere must be reconciled.

10.3 Record Maintenance

The Investigator must retain a comprehensive and centralized filing system of all clinical study-related documentation that is suitable for inspection by the Sponsor and representatives of regulatory authorities.

The Investigator must retain essential study documents (as specified in Section 8 of ICH-GCP and as required by the applicable regulatory requirements) until at least 2 years after the last approval of a marketing application. Patient files and other source data (including copies of protocols, CRFs, original reports of test results, agent-dispensing logs, correspondence, records of informed consent, and other documents pertaining to the conduct of the trial) must be kept for the maximum period of time permitted by the institution.

No trial document will be destroyed without prior written agreement between the Sponsor and the Investigator. Should the Investigator wish to assign the trial records to another party or move them to another location, written agreement must be obtained from the Sponsor.

The Principal Investigator shall take responsibility for maintaining adequate and accurate hard-copy source documents of all observations and data generated during this study, including any data clarification forms (DCF's) received from the Sponsor. Such documentation is subject to inspection by the Sponsor and the FDA or other Regulatory Authorities.

10.4 Study Center File Management

It will be the responsibility of the Principal Investigator to assure that the study file at the center is maintained. The study file for this protocol will contain, but will not be limited to, the information listed below:

- Investigator's Brochure (all versions provided during the study period.)
- Final study protocol.
- Protocol amendments (if applicable).
- Original informed consent form (blank).
- Revised informed consent forms and/or all addenda (if applicable).
- Copy of signed FDA Form(s) 1572
- Curricula Vitae and medical licenses of Principal Investigator and Subinvestigators.
- Financial Disclosure Form of Principal Investigator and Subinvestigators (if applicable).
- DHHS Number for IRB, or other documentation of IRB compliance with FDA regulation (US sites).
- Documentation of IRB/IEC approval of protocol, consent form, any protocol amendments and any consent form revisions.
- Annual IRB/IEC updates and approvals.
- All correspondence between the Principal Investigator, IRB/IEC and Sponsor or Sponsor's representative relating to study conduct.
- Copies of all 7-day and 15-day Safety Reports submitted to the Regulatory Authorities (provided by Sponsor) and IRB/IEC correspondence documenting their submission.
- Laboratory certifications.
- Normal laboratory value ranges for tests required by the protocol for all laboratories that are utilized.
- CRA monitoring log.
- List of signatures and Delegation of Authority for all study personnel
- Drug invoices for both receipt and return of study drug, as well as drug inventory/accountability records.

11. STATISTICAL ANALYSIS

A brief overview of the statistical analysis plan is presented below. Complete details of the planned analysis will be documented in a full Statistical Analysis Plan, which will be finalized before locking the study database.

11.1 Statistical Methods

Statistical analyses will be performed using Statistical Analysis System (SAS) software. Default estimation methods in version 9.4 of SAS are always used unless an alternative is specified below. In general, data summaries will include the mean, standard deviation, median, minimum and maximum values for continuous data; the median, 25th and 75th percentiles, minimum and maximum values for time-to-event endpoints; and the number and percentage of patients in each category for categorical data. Pointwise 95% confidence intervals (CI) will also be estimated for the mean (continuous data), median (time-to-event endpoints) or percentage of patients (categorical data).

Durations of time expressed in months (eg, times-to-event endpoints) will be reported in proportional “months” according to the formula

$$months = \frac{12}{365} \times \ days$$

Baseline value of a characteristic is defined as the last measured value prior to the first dose of study drug.

Statistical p-values will be truncated for display at the third digit following the decimal point. P-values less than 0.001 will be displayed as “<0.001.”

11.2 Sample Size

11.2.1 Sample Size in Stage 1

The sample size of 23 eligible patients for Stage 1 was selected because it allows estimation of the remission rate by means of a 90% confidence interval with maximum width of ±17%. Although this study does not follow a Simon 2-stage design, 23 patients is consistent with the sample size for Stage 1 of the Simon 2-stage minimax design with 80% power for testing the null hypothesis that the remission rate is 50% against a one-sided alternative at the 5% level of significance when the actual remission rate is 70%. In the Simon 2-stage design described, the study would continue to Stage 2 only if 13 patients achieve remission out of a maximum of 23 eligible patients. Therefore, this was selected as the requirement to proceed to Stage 2 of this study. However, the study may proceed to Stage 2 as soon as 13 patients achieve remission.

The primary objective of Stage 1 is to determine the CR rate in patients with relapsed or refractory AML with MCL-1 dependence of ≥***30***%. Thus a patient who leaves the study prior to providing a bone marrow sample adequate for assessing response to treatment will be replaced by another eligible patient.

11.2.2 Sample Size in Stage 2

The planned sample size of 56 patients (28 per treatment arm) in Stage 2 of this study provides 90% power to reject the null hypothesis of equal CR rates across treatment arms under the following conditions:

- CR rates of 70% and 30% for patients receiving ACM and CM, respectively
- One-sided 2.5% significance level
- Randomization based on a 1:1 patient allocation ratio (ACM to CM)

The primary objective of Stage 2 is to compare CR rates between patients with relapsed or refractory AML with MCL-1 dependence of ≥***30***% receiving 1 cycle of ACM treatment and those receiving 1 cycle of CM. Thus a patient who leaves the study prior to receiving one course of study treatment will be replaced by another eligible patient.

11.3 Analysis Populations

- **Intent-to-treat (ITT) patient population**: includes all patients enrolled into Stage 1; the exploratory arms; or randomized to receive study drug in Stage 2 regardless of whether they actually received study drug and regardless of whether evidence is found indicating they failed to meet study inclusion/exclusion criteria or had other protocol violations. When the ITT patient population is analyzed, Stage 2 patients are grouped according to their randomized treatment regardless of actual treatment received. The ITT patient population is the analysis population for all primary statistical inference and tests of hypotheses related to efficacy endpoints.

- **Safety patient population:** a subset of ITT patients who received at least one dose of study drug. When the safety patient population is analyzed, patients are grouped according to actual treatment received. ACM is considered a patient's "treatment received" in the safety patient population if alvocidib is administered at any time during the study. The safety patient population is the analysis population for all analyses of safety data.

- **Per-protocol patient population**: a subset of the safety patient population who either (a) have at least one response evaluation on study, or (b) die prior to their first scheduled response evaluation. However, patients are excluded from the per-protocol patient population if they have

an important protocol deviation, such as when evidence is found indicating they failed to meet any study inclusion/exclusion criterion. When the per-protocol patient population is analyzed, patients are grouped according to actual treatment received. ACM is considered a patient's "treatment received" in the per-protocol patient population if alvocidib is administered at any time prior to CR (or at any time during the study for patients never achieving CR). The per-protocol population is used for sensitivity (secondary, supportive) analyses of efficacy endpoints.

- **Response-assessment population:** a subset of the safety population who have at least one response evaluation on study. When the response-assessment patient population is analyzed, patients are grouped according to actual treatment received. ACM is considered a patient's "treatment received" in the response assessment patient population if alvocidib is administered at any time prior to CR (or at any time during the study for patients never achieving CR). The response-assessment population is the primary population for assessing the correlation between MCL-1 dependency and response to ACM and is used for sensitivity (secondary, supportive) analyses of efficacy endpoints.

11.4 Data Analyses

The overall significance level for this study will be 2.5% using one-sided hypothesis tests. Results from Stage 1 will be reported using descriptive statistics and confidence intervals. Primary statistical comparisons of efficacy results will use only data from Stage 2; however, data from Stage 1 patients will be combined with data from patients receiving ACM in Stage 2 for secondary efficacy analyses. Details of the analyses planned for this study are provided in the Statistical Analysis Plan under separate cover.

Stage 2 complete remission (CR) rates will be compared across treatment groups using the Cochran-Mantel-Haenszel general association test stratified by response to most recent induction therapy (refractory [persistent disease or CR duration <90 days], early relapse [CR duration 90 days to 1 calendar year], or late relapse [CR duration >1 calendar year but <24 months]). The same test will be applied to assess statistical significance of secondary response rate endpoints. Control of the overall 2.5% type-1 error rate for multiple comparisons will be achieved using the hierarchical closed test procedure.

Treatment-group distributions of time-to-event endpoints will be compared using the log-rank test stratified by response to most recent induction therapy.

Incidence rates of TEAEs will be summarized within treatment group at the MedDRA preferred term and primary system organ class levels. Similar summaries will be made for subsets of AEs such as (1) those judged by the Investigator to be related to study treatment, and (2) SAEs.

Other routine safety assessments (eg, clinical laboratory parameters and vital signs) will be summarized by treatment group using mean, standard deviation, median, minimum and maximum changes from baseline values.

A DSMB will monitor key outcomes from the study.

11.4.1 Demographics and Baseline Characteristics

Demographics and baseline characteristics will be summarized.

11.4.2 Efficacy Analyses

Response assessments in Stage 1 are defined by the International Working Group and 2010 ELN Criteria [20, 21]. The 2017 ELN criteria will be used to determine patient responses during Stage 2 [23] (*Appendix E*). The primary analysis for these response rates will be performed using data from all enrolled patients (intent-to-treat, ITT). In the ITT analysis, patients withdrawing from the study for any reason prior to a response assessment will be classified as a nonresponder. A secondary analysis of per protocol patients will also be performed.

Primary Efficacy Endpoint

- Complete Remission (CR) rate = Percentage of patients achieving CR

Secondary Efficacy Endpoints

- Combined CR Rate = Percentage of patients achieving a CR, CRi or CRp[†]
- Combined Response Rate = Percentage of patients achieving a CR, CRi, CRp[†], or PR
- Overall Survival (OS) = Time from randomization (Day 1) until death from any cause
- Event-free Survival (EFS) = Time from randomization (Day 1) until (a) treatment failure, (b) relapse after CR, or (c) death from any cause, whichever occurs first, censored at 2 years
- Rate of Stem Cell Transplantation = Percentage of patients proceeding directly to stem cell transplantation

[†]Note that CRp has been retained in definitions for Combined CR Rate and Combined Remission Rate because Stage-1 patients were assessed using the IWG criteria. CRp will not be used for assessing response in Stage-2 patients and, therefore, will not be included in the definitions of Combined CR Rate and Combined Remission Rate in Stage 2.

The CR rate in patients failing CM and crossing over to receive ACM will also be determined.

Secondary endpoints described for Stage 1/Stage 2 will also be assessed.

Additional exploratory analyses may be performed to assist the sponsor in planning future studies.

Without taking appropriate measures, testing multiple efficacy parameters inflates the overall significance level since the probability of making a type-1 error in at least one from a set of hypothesis tests is greater than the probability of making a type-1 error in any single test. The hierarchical closed test procedure will be used to maintain the overall 2.5% significance level.

The order of efficacy endpoints for the hierarchical closed test procedure is:

1. CR rate
2. Combined CR rate
3. Combined Response Rate
4. OS
5. EFS
6. Stem cell transplant rate

Each efficacy endpoint will be tested at a one-sided 2.5% significance level using the statistical procedures described above. CR rate, the primary efficacy endpoint, will be declared statistically significant if its test p-value ≤0.025. A secondary efficacy endpoint will be declared statistically significant only if its test p-value ≤0.025 and all efficacy endpoints preceding it in the hierarchy are statistically significant. In other words, declaration of statistical significance for efficacy endpoints will advance in order down the hierarchy only until a p-value >0.025 is found.

In addition, although there will be primary and supportive analytical methods for each endpoint in the hierarchy (supportive methods will be documented in the formal Statistical Analysis Plan), only the p-value from the primary analysis will be used for deciding statistical significance. Supportive (sensitivity) analyses are to demonstrate the robustness of primary results to slight modifications to how endpoints are defined and/or analyzed.

Declarations of statistical significance will not be advanced for endpoints not included in the hierarchy of the closed test procedure.

11.4.3 Pharmacodynamic Endpoint Analyses

Summary of baseline biomarker values will include within– and between–treatment-arm descriptive statistics. A logistic regression model will be fit to examine the relationship between baseline MCL-1 dependence and the independent binary variable achievement of CR during the study. The full model will include effects for baseline MCL-1 dependence, treatment arm, and the two-way interaction between baseline MCL-1 dependence and treatment arm. In addition, area under the curve (AUC) and 95% CI will be calculated (by treatment

arm) for the trapezoidal receiver operating characteristic curve. This value will quantify the ability of MCL-1 dependence to predict CR.

11.4.4 Safety Analyses

The following safety analyses will be performed on all patients who receive at least one dose of study drug.

- **30- and 60-day Mortality** = Estimates of 30- and 60-day mortality and their pointwise 95% CIs will be derived from Kaplan-Meier curves for OS by treatment arm
- **Adverse Events** = Reported AE terms will be mapped to MedDRA preferred terminology. All reported events will appear in AE listings, however only TEAEs will be summarized. A TEAE is an AE that starts or increases in severity any time after the first administration of any study drug up to 30 days following the last administration of any study drug. AE severity will be rated by the investigator according to NCI CTCAE version 4.03 in Stage 1 and version 5.0 in Stage 2 (see *Appendix C*).
- **Clinical Laboratory Assessments** = Laboratory test results measured on a continuous scale and changes from baseline values will be summarized by visit and within treatment arm using mean, standard deviation, median, minimum and maximum values. Categorical test results will be summarized within treatment arm using shift tables. Additionally, for tests where NCI CTCAE, version 4.03 in Stage 1 and version 5.0 in Stage 2 (see *Appendix C*), severity criteria are specified, NCI CTCAE severity grades will be summarized in shift tables.
- **Vital Signs** = Vital signs and changes from baseline values will be summarized by visit and within treatment arm using mean, standard deviation, median, minimum and maximum values.

12. PROTOCOL AMENDMENTS

Any permanent change to the protocol must be handled as a protocol amendment. Protocol amendments will be written by the Sponsor. All protocol amendments must be submitted in writing to the Institutional Review Board (IRB)/Independent Ethics Committee (IEC), and the Principal Investigator must await IRB/IEC approval of the amendments before implementing the changes. However, a protocol change that is intended to eliminate an apparent immediate hazard to patients may be implemented immediately and the IRB/IEC is to be notified within five (5) days. The Sponsor should also be notified by telephone as soon as possible, ideally before the amendment is implemented and definitely within 5 days. The Sponsor will submit protocol amendments to the Regulatory Authorities.

When an amendment to the protocol substantially alters the study design and/or increases the potential risk to the patient, the currently approved written informed consent form will require similar modification and IRB/IEC approval. In such cases, repeat written informed consent will be obtained from patients currently enrolled in the study before expecting continued participation.

13. MONITORING

Prior to enrolling any participants, a study initiation visit, including protocol training, will be conducted for the study center. A Study Manual of Procedures will be provided to each clinical site. A record of site personnel training will be maintained by the site onsite training logs.

Clinical Research Associates (CRAs) and other applicable personnel will receive training prior to study initiation about the disease, applicable Standard Operating Procedures (SOPs), the protocol and other study-specific items. Team organization, communication, and operational issues will also be discussed.

The conduct of the study will be closely monitored by representatives (Clinical Research Associates "CRAs" or study monitors) of the Sponsor or designee, to verify adherence to the Protocol, ICH GCP guidelines, and applicable regulations. The CRA will verify electronic CRF (eCRF) entries by comparing them with Sponsor/site-generated source documents, hospital, clinic, office and/or study records which will be made available for this purpose. CRAs will monitor the study as outlined in the Monitoring Plan prepared for the study.

During the study, CRAs will visit the clinical sites to assess and assure satisfactory enrollment rate, data recording, and maintenance of required regulatory documentation, drug accountability, and compliance with the protocol. CRAs will also be able to monitor the data remotely. The Investigator will ensure that all requested materials, including subject charts, eCRFs, source documents, laboratory records, and drug inventory records, will be available to the CRA. At the end of the study, a closeout visit will be performed.

The Investigator will allow Sponsor's representatives, designee and/or and any regulatory agency to have direct access to all study records, eCRFs, corresponding subject medical records, test product dispensing records and test product storage area, and any other documents considered source documentation. The Investigator also agrees to assist the representative, if required.

14. AUDITING

The study is conducted under the sponsorship of Tolero Pharmaceuticals, Inc, in compliance with the applicable international and local regulatory requirements as well as applicable ICH guidelines, Declarations of Helsinki (1964, 1975, 1983, 1989, 1996, 2000, 2002, 2004, 2008, 2013) and in respect of the Sponsor or designee's SOPs for study conduct and monitoring.

Audits may be carried out by Sponsor representatives, and inspection may be performed by regulatory authorities' inspectorate or IRBs/IECs before, during, or after the study. The Investigator will allow and assist Sponsor's representatives and any regulatory agency to have direct access to all study records, eCRFs, subject medical records, study product dispensing records and study product storage area, study facilities, and any other documents considered source documentation.

For the Audit(s) performed by, or on behalf of, Sponsor's auditors, audit certificate(s) will be provided by Quality Assurance.

15. ETHICS AND RESPONSIBILITY

15.1 Principal Investigator's Responsibilities

The PI shall ensure that all work and services described herein, or incidental to those described herein, shall be conducted in accordance with the highest standards of GCP. The PI shall administer the investigational drug only to patients under his/her personal supervision, or under the supervision of any Sub-Investigator(s) responsible to him/her, who are identified on the Form FDA 1572/Regulatory Authorities approval form. The PI will provide copies of the study protocol, amendments, and investigational brochure to all Sub-Investigators, Pharmacists, or other staff responsible for study conduct.

With the exception of eliminating an immediate hazard to a subject, the Investigator should not deviate from the protocol or implement any changes without written prior approval from Sponsor and prior review and documented approval/favorable opinion from the IRB/IEC of an amendment.

Change(s) which involve(s) only logistical or administrative changes are authorized. The Investigator should document and explain any deviation from the protocol.

The Investigator is responsible for adequate and safe medical care of subjects during the trial and for ensuring that appropriate medical care and relevant follow-up procedures are maintained after the trial. Any additional data from these follow-up procedures must be documented and available to Sponsor who will determine whether or not the data need to be documented in the CRFs.

15.2 Informed Consent

It is the ethical and legal responsibility of the PI to ensure that each patient considered for inclusion in this study is given a full explanation of the protocol, in a language in which the patient is fluent, and in which the patient will clearly understand. This shall be documented on a written informed consent form, which shall be approved by the same IRB/IEC responsible for approval of this protocol. Each informed consent form shall include the elements required by local regulations. The Sponsor will draft this document in consultation with the PI. The PI agrees to obtain written approval of the consent form from the Sponsor prior to submission to the IRB/IEC.

Once the appropriate essential information has been provided to the patient and fully explained by the PI (or his/her qualified Designee) and it is felt that the patient understands the implications of participating in the study, the IRB/IEC-approved consent form shall be signed by the patient, a witness (when appropriate) and the PI. Written informed consent will be obtained from each patient prior to any study-related procedures (including any pre-treatment procedures) that are performed. The patient shall be given a copy of the informed consent form when signed; the original shall be kept on file by the PI and a second copy shall be placed in the patient's medical chart.

15.3 Institutional Review Board/Independent Ethics Committee

This protocol and all amendments will be reviewed and approved by the Institutional or Independent Review Board(s) or Independent Ethics Committee(s) charged with this responsibility at the study center. Notification in writing of approval must come from the Chairman or the Secretary of the IRB/IEC meeting minutes where this protocol and associated informed consent form were discussed. The PI shall not participate in the decision, and, if an IRB/EC member, the written approval must indicate such non-participation. The PI shall submit status reports to the IRB/IEC no less frequently than annually (when applicable).

The IRB/IEC must be notified by the PI in writing of the interruption and/or completion of the study; the PI must promptly report to the IRB/IEC all changes in research (protocol amendments) and will not make such changes without IRB/IEC approval, except where necessary to eliminate apparent immediate hazards to human patients. In these cases, the IRB/IEC must be notified within five days of the change. The PI will promptly report to the IRB/IEC all unanticipated problems involving risk to patients or others. The PI is required to maintain an accurate and complete record of all written correspondence to, and received from the IRB/IEC, and must agree to share all such documents and reports with the Sponsor.

16. CONFIDENTIALITY

The existence of this clinical study is confidential and should not be discussed with persons outside of the study. The Investigator shall hold confidential, and not disclose directly or indirectly to any third party other than those persons involved in the study who have a need to know, the protocol, the data arising out of the study, and any other information related to the study or to Tolero's products or research programs that is provided by Tolero to you (the "Confidential Information"). All such persons must be instructed not to further disseminate this information to others. Investigator shall not use the Confidential Information for any purpose other than the study. The foregoing obligations of confidence and non-use assumed by you shall not apply to: (a) information which at the time of disclosure is in the public domain; (b) information which thereafter lawfully becomes part of the public domain other than through disclosure by or through you; (c) information which, as evidenced by your written records, was known by you prior to Tolero's disclosure; (d) information which is lawfully disclosed to you by a third party not under any obligation of confidence to Tolero; or (e) information which is required to be disclosed by law or government regulatory agency, provided reasonable advance notice of such disclosure is given to Tolero.

All information generated in this study must be considered highly confidential and must not be disclosed to any persons not directly concerned with the study, without written permission from the Sponsor. However, authorized drug regulatory officials and the Sponsor's representatives will be allowed full access to the records.

Patients will be identified only by initials and assigned a patient number. Their full names may, however, be made known to a drug regulatory agency or other authorized official if necessary.

All data and discoveries arising out of the study, patentable or non-patentable, shall be the sole property of Tolero, Inc.

In signing this protocol, Investigator agrees to the release of the data from this study and acknowledges the above confidentiality and publication policy. The provisions of this Statement shall survive the completion of the study.

Clinical information will not be released without the written permission of the patient, except as necessary for monitoring by the Sponsor or the Regulatory Authorities, or as required by law.

17. NONPROTOCOL RELATED RESEARCH

The Sponsor has a legal responsibility to report fully to regulatory authorities all the results of administration of investigational drugs. No investigative procedures other than those in this protocol shall be undertaken on the enrolled patients without the agreement of the IRB/IEC and the Sponsor's medical monitor.

18. PUBLICATIONS

The publication policy for the study will be described in the clinical study agreement. To avoid disclosures that could jeopardize proprietary rights, the investigator agrees to give Tolero Pharmaceuticals, Inc, the right to review all manuscripts, abstracts, and presentations related to this study *prior* to their submission for publication or presentation. Tolero may use these data now and in the future for presentation or publication at Tolero's discretion or for submission to government regulatory agencies.

Authorship among Investigators generally will be based on the extent of significant contribution, including scientific and clinical, to the publication.

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APPENDIX A – SCHEDULE OF ACTIVITIES – STAGE 1, Cycle 1 (ACM only)

	SCREENING		CYCLE 1					
TESTS/PROCEDURES	Within 2 Wks Prior to 1st Dose	Within 72 Hrs Prior to 1st Dose	At Least 10 Hrs Prior to 1st Dose	Just Prior to 1st Dose	Dosing	Daily During Chemo (Days 1- 9)	Weekly - Post Treatment	At Hematologic Recovery or Day 45 (±2)[bb]
Prescreening – Marrow aspirate for MCL-1 dependence, disease history	X							
Obtain Study Informed Consent(s)	X							
Medical history[a]	X							
Physical examination+weight (kg)	X		X				X[y]	
Height (cm)	X							
ECOG performance status	X		X				X	
Vital signs[b]	X			X[s]			X	
Bone marrow biopsy/aspirate	X[k]							X[z]
Cytogenetic profiling	X[k]							
12-lead ECG	X							
ECHO or MUGA	X							
Chest radiograph[c]	X							
Concomitant medications	X[l]	X[m]	X[o]	X[m]	X[u]	X[u]	X[u]	X[u]
Pregnancy test[d]		X		X[t]				
Hematology[e]		X				X	X	
Full serum chemistry panel[f]		X						
Review Inclusion/Exclusion Criteria		X[n]						
Urinalysis[g]		X						
Determination of BSA			X					
Pretreatment: IV hydration, allopurinol, oral phosphate binder			X[p,q,r]					
Monitor urine output frequently[h]			X		X	X		
Tumor lysis labs[i]				X	X[v,w]	X[v,w]	X	
Coagulation: fibrinogen				X	X[x]	X[x]	X[x]	
Treatment with ACM					X[v,w]	X[v,w]		
Prophylactic antibiotics and antivirals, steroid eye drops, antifungals					X			
Assessment of AEs[j]					X	X	X	X[aa]

Notes:

a Collect and document a complete medical history including pathological confirmed diagnosis of AML by WHO criteria and documentation of all other measures of disease and disease symptoms (eg, extramedullary disease)
b Vital signs to include: body temperature, heart rate, systolic and diastolic blood pressure
c Required if not performed/obtained within 30 days prior to anticipated first dose.
d Collect urine or serum sample for β-hCG pregnancy test from females of child-bearing potential
e *See Appendix D* – Laboratory Tests, Hematology
f *See Appendix D* – Laboratory Tests, Full Serum Chemistry
g *See Appendix D* – Laboratory Tests, Urinalysis
h Diligent monitoring of urine output **frequently** to ensure that it equals fluid input. If input is greater than output by 10%, administration of diuretics is encouraged. Replacement of excessive fluid losses, including from diarrhea, should be done unless otherwise clinically indicated. Test for presence of *C. difficile* infection at first instance of diarrhea (refer to *Section 4.6.1.2* for additional guidance).
i *See Appendix D* – Laboratory Tests, Tumor Lysis Labs
j Toxicities will be assessed according to the NCI CTCAE, version 4.03 in Stage 1 (*see Appendix C*). When the NCI CTCAE grade is not available, the investigator will use the following toxicity grading: mild, moderate, severe, life-threatening or fatal
k Bone marrow biopsy and/or aspirate required for diagnosis, cytogenetic profiling, and BH3 profiling. If the initial bone marrow biopsy and/or aspirate is nonproductive or not diagnostic, the procedure must be repeated. (If an aspirate with cytogenetics was performed as part of the BH3 profiling and it was done within 2 weeks of the first dose, then it does not need to be repeated.) At screening, 6 to 8 bone marrow slides will be prepared and sent to the Sponsor if the patient is enrolled. Details regarding the collection and shipping of these slides is provided in the Study Manual.
l Including all nonprescription medications and nutritional supplements taken within the past 2 weeks
m Including all prescription and nonprescription medications and nutritional supplements
n Review all inclusion and exclusion criteria to determine if patient has met all eligibility criteria for enrollment into the study.
o Including all nonprescription medications and nutritional supplements taken since screening
p Mandatory IV hydration with 0.45% NaCl (or similar hydration fluid per institutional standard) sterile solution at 100 cc/hour for at least 10 hours prior to initiation of first dose during Cycle 1 (optional for subsequent cycles). If, by Day 4, there is no evidence of tumor lysis syndrome, the hydration rate can be reduced to a maintenance level.
q Mandatory allopurinol orally each day of dosing for first cycle to be started at same time as initiation of IV hydration.
r Mandatory oral phosphate binder to be started at the same time as initiation of IV hydration, unless contraindicated.
s Measured 5-15 minutes prior to the initiation of infusion following a 5-minute rest
t Required if screening pregnancy test was performed greater than 72 hrs prior
u To include only those medications (prescription, nonprescription, nutritional supplements) administered in conjunction with an AE.
v For patients at **high risk** for TLS: See *Section 4.6.1.1* for TLS labs collection schedule.
w For patients **NOT at high risk** for TLS: See *Section 4.6.1.1* for TLS labs collection schedule.
x Only required if clinically indicated
y Abbreviated physical examination (AE- or symptom-directed exam).
z Bone marrow biopsies and/or aspirates will be performed at hematologic recovery (ie, absolute neutrophil count (ANC) >1000 µL and platelet count >100,000 µL) or on Day 45 (±2), whichever occurs first. If the bone marrow biopsy and/or aspirate is nonproductive or not diagnostic, the procedure must be repeated within 7 days. In addition, 6 to 8 bone marrow slides will be prepared and sent to the Sponsor. Details regarding the collection and shipping of these slides is provided in the Study Manual.
aa Including tumor lysis clinical symptoms
bb An End of Study visit is to be performed at time of patient withdrawal [See SCHEDULE OF ACTIVITIES – STAGE 1, Cycles 2 - 4 (ACM only) for details of required assessments]

APPENDIX A – SCHEDULE OF ACTIVITIES – STAGE 1, Cycles 2 - 4 (ACM only)

TESTS/PROCEDURES	CYCLES 2+[a]						End of Study	Follow up[v]
	At Least 10 Hrs Prior to 1st Dose	Just Prior to 1st Dose	Dosing	Daily During Chemo (Days 1- 9)	Weekly - Post Treatment	At Hematologic Recovery or Day 45 (±2)		
Pretreatment: IV hydration, allopurinol, oral phosphate binder	X[k,l,m]							
Monitor urine output frequently[b]	X	X	X	X				
ECHO or MUGA	X[n]						X[p]	
Physical examination+weight (kg)		X			X[r]		X[r,t]	
Determination of BSA		X						
Vital signs[c]		X[o]			X		X	
ECOG performance status		X			X		X	
Hematology[d]		X	X	X	X		X	
Tumor lysis labs[e]		X	X	X	X			
Coagulation: fibrinogen		X[p]	X[p]	X[p]	X[p]		X[p]	
Pregnancy test[f]		X						
Concomitant medications[g]		X	X	X	X	X	X	
Treatment with ACM			X[q]	X[q]				
Prophylactic antibiotics and antivirals, steroid eye drops, antifungals			X					
Assessment of AEs[h]			X	X	X	X	X	
Bone marrow biopsy/aspirate						X[s]	X[s,u]	
Full serum chemistry panel[i]							X	
Urinalysis[j]							X	
12-lead ECG							X[p]	
Telephone call to patient								X

Notes:

a Patients who achieve CR, CRi, CRp, or PR after the first cycle (completion of all doses) may receive up to 3 additional optional cycles of treatment. After completing the first cycle of treatment, continued use of mitoxantrone is optional. Mitoxantrone must be omitted from subsequent cycles if the patient's lifetime daunorubicin equivalent exceeds 460 mg/m^2 (see *Appendix G* for conversion table) or the LVEF drops below 45%. Patients not

demonstrating evidence of CR, CRi, CRp, or PR after the first cycle of treatment will be considered for removal from the study, although with permission of the Medical Monitor, induction treatment may continue if clinically indicated and provided there is no evidence of toxicity ≥NCI CTCAE grade 4.

b If pretreatment to manage/treat tumor lysis syndrome is clinically indicated in subsequent cycles, must monitor urine output **frequently** to ensure that it equals fluid input. If input is greater than output by 10%, administration of diuretics is encouraged. Replacement of excessive fluid losses, including from diarrhea, should be done unless otherwise clinically indicated. Test for presence of *C. difficile* infection at first instance of diarrhea (refer to *Section 4.6.1.2* for additional guidance).

c Vital signs to include: body temperature, heart rate, systolic and diastolic blood pressure

d See *Appendix D* – Laboratory Tests, Hematology

e See *Appendix D* – Laboratory Tests, Tumor Lysis Labs

f See *Appendix D* – Laboratory Tests, Urinalysis

g To include only those medications (prescription, nonprescription, nutritional supplements) administered in conjunction with an AE.

h Toxicities will be assessed according to the NCI CTCAE, version 4.03 in Stage 1 (see *Appendix C*). When the NCI CTCAE grade is not available, the investigator will use the following toxicity grading: mild, moderate, severe, life-threatening or fatal

i See *Appendix D* – Laboratory Tests, Full Serum Chemistry

j Urinalysis to include: color, specific gravity, pH, bilirubin, ketones, glucose, hemoglobin, blood, protein, urobilinogen, nitrites, WBCs, RBCs, casts, crystals, bacteria

k If clinically indicated, begin IV hydration with 0.45% NaCl (or similar hydration fluid per institutional standard) sterile solution at 100 cc/hour at least 10 hours prior to initiation of first dose. If, by Day 4, there is no evidence of tumor lysis syndrome, the hydration rate can be reduced to a maintenance level.

l If clinically indicated, begin allopurinol orally each day of dosing at same time as initiation of IV hydration.

m If clinically indicated, begin oral phosphate binder at the same time as initiation of IV hydration, unless contraindicated.

n ECHO or MUGA scan to be performed before each dose of mitoxantrone. If mitoxantrone is omitted after Cycle 1, the ECHO or MUGA scan can also be omitted.

o Measured 5-15 minutes prior to the initiation of infusion following a 5-minute rest

p Only required if clinically indicated

q Continued use of mitoxantrone is optional. Mitoxantrone must be omitted from subsequent cycles if the patient's lifetime daunorubicin equivalent exceeds 460 mg/m^2 (see *Appendix G* for conversion table) or the LVEF drops below 45%.

r Abbreviated physical examination (AE- or symptom-directed exam).

s Bone marrow biopsies and/or aspirates will be performed at hematologic recovery (ie, absolute neutrophil count (ANC) >1000 µL and platelet count >100,000 µL) or on Day 45 (±2), whichever occurs first. If the bone marrow biopsy and/or aspirate is nonproductive or not diagnostic, the procedure must be repeated within 7 days. At time of response assessment and end-of-study visit, 6 to 8 bone marrow slides will be prepared and sent to the Sponsor. Details regarding the collection and shipping of these slides is provided in the Study Manual.

t Including other measures of disease and disease symptoms, eg, extramedullary disease

u If not done in preceding 30 days

v Patients will be contacted by telephone to assess for date of death, date of stem cell transplant, date of relapse, or continued remission for two years: Year 1: Phone calls monthly beginning the month after the patient completes the end-of-study assessments to 12 months after Day of Randomization regardless of how many cycles a patient receives (Year 1 = Day of Randomization plus 12 months); Year 2: Phone calls every other month during Year 2 (months 14 to 24 months after Day of Randomization).

APPENDIX A – SCHEDULE OF ACTIVITIES – STAGE 2, Cycle 1 (ACM vs CM)

	SCREENING		CYCLE 1					
TESTS/PROCEDURES	Within 2 Wks Prior to 1st Dose	Within 72 Hrs Prior to 1st Dose	At Least 10 Hrs Prior to 1st Dose	Just Prior to 1st Dose	Dosing	Daily During Chemo (Days 1- 9 for ACM; Days 1- 4 for CM)	Weekly - Post Treatment	At Hematologic Recovery or Day 45 (±2)[bb]
Prescreening – Marrow aspirate for MCL-1 dependence, disease history	X							
Obtain Study Informed Consent(s)	X							
Medical history[a]	X							
Physical examination+weight (kg)	X		X				X[y]	
Height (cm)	X							
ECOG performance status	X		X				X	
Vital signs[b]	X			X[r]			X	
Bone marrow biopsy/aspirate	X[k]							X[z]
Cytogenetic profiling	X[k]							
12-lead ECG	X							
ECHO or MUGA	X							
Chest radiograph[c]	X							
Pregnancy test[d]	X	X		X[s]				
Concomitant medications	X[l]	X	X[n]	X[t]	X[u]	X[u]	X[u]	X[u]
Hematology[e]		X				X	X	
Full serum chemistry panel[f]		X						
Urinalysis[g]		X						
Review Inclusion/Exclusion Criteria		X[m]						
Patients randomized to ACM or CM		X			X[v,w]	X[v,w]		
Determination of BSA			X					
Pretreatment: IV hydration, allopurinol, oral phosphate binder			X[o,p,q]					
Monitor urine output frequently[h]			X		X	X		
Tumor lysis labs[i]				X	X[v,w]	X[v,w]	X	
Coagulation: fibrinogen				X	X[x]	X[x]	X[x]	
Prophylactic antibiotics and antivirals, steroid eye drops, antifungals					X			
Assessment of AEs[j]					X	X	X	X[aa]

Notes:

a Collect and document a complete medical history including pathological confirmed diagnosis of AML by WHO criteria and documentation of all other measures of disease and disease symptoms (eg, extramedullary disease)
b Vital signs to include: body temperature, heart rate, systolic and diastolic blood pressure
c Required if not performed/obtained within 30 days prior to anticipated first dose.
d Collect urine or serum sample for β-hCG pregnancy test from females of child-bearing potential
e See *Appendix D* – Laboratory Tests, Hematology
f See *Appendix D* – Laboratory Tests, Full Serum Chemistry
g See *Appendix D* – Laboratory Tests, Urinalysis
h Diligent monitoring of urine output **frequently** to ensure that it equals fluid input. If input is greater than output by 10%, administration of diuretics is encouraged. Replacement of excessive fluid losses, including from diarrhea, should be done unless otherwise clinically indicated.
i See *Appendix D* – Laboratory Tests, Tumor Lysis Labs
j Toxicities will be assessed according to the NCI CTCAE, version 5.0 in Stage 2 (see *Appendix C*). When the NCI CTCAE grade is not available, the investigator will use the following toxicity grading: mild, moderate, severe, life-threatening or fatal.
k Bone marrow biopsy and/or aspirate required for diagnosis, cytogenetic profiling, and BH3 profiling. If the initial bone marrow biopsy and/or aspirate is nonproductive or not diagnostic, the procedure must be repeated. (If an aspirate with cytogenetics was performed as part of the BH3 profiling and it was done within 2 weeks of the first dose, then it does not need to be repeated). At screening, 6 to 8 bone marrow slides will be prepared and sent to the Sponsor if the patient is enrolled. Details regarding the collection and shipping of these slides is provided in the Study Manual.
l Including all nonprescription medications and nutritional supplements taken within the past 2 weeks
m Review all inclusion and exclusion criteria to determine if patient has met all eligibility criteria for enrollment into the study.
n Including all nonprescription medications and nutritional supplements taken since screening
o Mandatory IV hydration with 0.45% NaCl (or similar hydration fluid per institutional standard) sterile solution at 100 cc/hour for at least 10 hours prior to initiation of first dose during Cycle 1 (optional for subsequent cycles) in both treatment arms. If, by Day 4, there is no evidence of tumor lysis syndrome, the hydration rate can be reduced to a maintenance level.
p Mandatory allopurinol orally each day of dosing for first cycle to be started at same time as initiation of IV hydration in both treatment arms.
q Mandatory oral phosphate binder to be started at the same time as initiation of IV hydration in both treatment arms, unless contraindicated.
r Measured 5-15 minutes prior to the initiation of infusion following a 5-minute rest
s Required if screening pregnancy test was performed greater than 72 hrs prior
t Including all nonprescription medications and nutritional supplements
u To include only those medications (prescription, nonprescription, nutritional supplements) administered in conjunction with an AE.
v For patients at **high risk** for TLS: See *Section 4.6.1.1* for TLS labs collection schedule.
w For patients **NOT at high risk** for TLS: See *Section 4.6.1.1* for TLS labs collection schedule.
x Only required if clinically indicated
y Abbreviated physical examination (AE- or symptom-directed exam).
z Bone marrow biopsies and/or aspirates will be performed at hematologic recovery or on Day 45 (±2), whichever occurs first. If the bone marrow biopsy and/or aspirate is nonproductive or not diagnostic, the procedure must be repeated within 7 days. At time of response assessment, 6 to 8 bone marrow slides will be prepared and sent to the Sponsor. Details regarding the collection and shipping of these slides is provided in the Study Manual.
aa Including tumor lysis clinical symptoms
bb An End of Study visit is to be performed at time of patient withdrawal [See SCHEDULE OF ACTIVITIES – STAGE 2, Cycles 2 - 4 (ACM vs CM) for details of required assessments]

APPENDIX A – SCHEDULE OF ACTIVITIES – STAGE 2, Cycles 2 - 4 (ACM vs CM)

TESTS/PROCEDURES	CYCLES 2+[a]						End of Study	Follow Up[v]
	At Least 10 Hrs Prior to 1st Dose	Just Prior to 1st Dose	Dosing	Daily During Hospitalization for Chemo	Weekly - Post Treatment	At Hematologic Recovery or Day 45 (±2)		
Pretreatment: IV hydration, allopurinol, oral phosphate binder	X[k,l,m]							
Monitor urine output frequently[b]	X	X	X	X				
ECHO or MUGA	X[n]						X[p]	
Physical examination+weight (kg)		X			X[r]		X[r,t]	
Determination of BSA		X						
Vital signs[c]		X[o]			X		X	
ECOG performance status		X			X		X	
Hematology[d]		X	X	X	X		X	
Tumor lysis labs[e]		X	X	X	X			
Coagulation: fibrinogen		X[p]	X[p]	X[p]	X[p]		X[p]	
Pregnancy test[f]		X						
Concomitant medications[g]		X	X	X	X	X	X	
Treatment with ACM or CM			X[q]	X[q]				
Prophylactic antibiotics and antivirals, steroid eye drops, antifungals			X					
Assessment of AEs[h]			X	X	X	X	X	
Bone marrow biopsy/ aspirate						X[s]	X[s,u]	
Full serum chemistry panel[i]							X	
Urinalysis[j]							X	
12-lead ECG							X[p]	
Telephone call to patient								X

Notes:

a Mitoxantrone must be omitted from subsequent cycles if the patient's lifetime daunorubicin equivalent exceeds 460 mg/m^2 *(see Appendix G* for conversion table) or the LVEF drops below 45%. Patients not demonstrating evidence of CR, CRi, CRp, or PR after the first cycle of treatment will be considered for removal from the study, although with permission of the Medical Monitor, induction treatment may continue if clinically indicated and provided there is no evidence of toxicity ≥NCI CTCAE grade 4. However, patients randomized to CM with progressive disease or no response after

1 cycle of CM may cross over to receive ACM. In addition, patients randomized to CM with the best response of a PR after 2 cycles of CM may also cross over to receive ACM. Patients who cross over to ACM may receive up to a *combined* total (CM + ACM) of 4 cycles *(*See *Section 4.5.3.4)*

b If pretreatment to manage/treat tumor lysis syndrome is clinically indicated in subsequent cycles, must monitor urine output **frequently** to ensure that it equals fluid input. If input is greater than output by 10%, administration of diuretics is encouraged. Replacement of excessive fluid losses, including from diarrhea, should be done unless otherwise clinically indicated.

c Vital signs to include: body temperature, heart rate, systolic and diastolic blood pressure

d See *Appendix D* – Laboratory Tests, Hematology

e See *Appendix D* – Laboratory Tests, Tumor Lysis Labs

f Collect urine or serum sample for β-hCG pregnancy test from females of child-bearing potential

g To include only those medications (prescription, nonprescription, nutritional supplements) administered in conjunction with an AE.

h Toxicities will be assessed according to the NCI CTCAE, version 5.0 in Stage 2 (see *Appendix C*). When the NCI CTCAE grade is not available, the investigator will use the following toxicity grading: mild, moderate, severe, life-threatening or fatal

i See *Appendix D* – Laboratory Tests, Full Serum Chemistry

j See *Appendix D* – Laboratory Tests, Urinalysis

k If clinically indicated, begin IV hydration with 0.45% NaCl (or similar hydration fluid per institutional standard) sterile solution at 100 cc/hour at least 10 hours prior to initiation of first dose in both treatment arms.-If, by Day 4, there is no evidence of tumor lysis syndrome, the hydration rate can be reduced to a maintenance level.

l If clinically indicated, begin allopurinol orally each day of dosing at same time as initiation of IV hydration in both treatment arms.

m If clinically indicated, begin oral phosphate binder at the same time as initiation of IV hydration in both treatment arms, unless contraindicated.

n ECHO or MUGA scan to be performed before each dose of mitoxantrone. If mitoxantrone is omitted after Cycle 1, the ECHO or MUGA scan can also be omitted.

o Measured 5-15 minutes prior to the initiation of infusion following a 5-minute rest

p Only required if clinically indicated

q Continued use of mitoxantrone is optional. Mitoxantrone must be omitted from subsequent cycles if the patient's lifetime daunorubicin equivalent exceeds 460 mg/m^2 *(*see *Appendix G* for conversion table) or the LVEF drops below 45%.

r Abbreviated physical examination (AE- or symptom-directed exam).

s Bone marrow biopsies and/or aspirates will be performed at hematologic recovery (ie, absolute neutrophil count (ANC) >1000 µL and platelet count >100,000 µL) or on Day 45 (±2), whichever occurs first. If the bone marrow biopsy and/or aspirate is nonproductive or not diagnostic, the procedure must be repeated within 7 days. At time of response assessment and end-of-study visit, 6 to 8 bone marrow slides will be prepared and sent to the Sponsor. Details regarding the collection and shipping of these slides is provided in the Study Manual.

t Including other measures of disease and disease symptoms, eg, extramedullary disease

u If not done in preceding 30 days

v Patients will be contacted by telephone to assess for date of death, date of stem cell transplant, date of relapse, or continued remission for two years: Year 1: Phone calls monthly beginning the month after the patient completes the end-of-study assessments to 12 months after Day of Randomization regardless of how many cycles a patient receives (Year 1 = Day of Randomization plus 12 months); Year 2: Phone calls every other month during Year 2 (months 14 to 24 ~~months~~ after Day of Randomization).

APPENDIX B – ECOG PERFORMANCE STATUS SCALE

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

Oken MM, Creech RH, Tormey DC, et al. Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 1982;5:649-655.
Available at: http://www.ecog.org/general/perf_stat.html

*The ECOG Performance Status is in the public domain therefore available for public use. To duplicate the scale, please cite the reference above and credit the Eastern Cooperative Oncology Group, Robert Comis, MD, Group Chair.

APPENDIX C – NCI COMMON TERMINOLOGY CRITERIA FOR ADVERSE EVENTS

View the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) v4.03 (for Stage 1) and v5.0 (for Stage 2) electronically at the following Web sites:

https://evs.nci.nih.gov/ftp1/CTCAE/CTCAE_4.03/CTCAE_4.03_2010-06-14_QuickReference_8.5x11.pdf

https://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/CTCAE_v5_Quick_Reference_8.5x11.pdf

The Study Manual includes copies of both versions of the NCI CTCAE.

APPENDIX D – LABORATORY TESTS

Hematology	• CBC with manual differential *Note: A manual differential is the preferred method and is required on each day that the assessment is done. Automated differentials may be used for subsequent differentials performed on the same day.*	• Platelet Count
Full Serum Chemistry	• Blood urea nitrogen (BUN) • Phosphorus • Magnesium • Lactate dehydrogenase (LDH) • Creatinine • Uric acid • Total protein • Albumin • Calcium • Glucose	• Total bilirubin • Alkaline phosphatase (ALP) Aspartate aminotransferase (AST) • Alanine aminotransferase (ALT) • Electrolytes o Sodium o Potassium o Chloride o Carbon dioxide (bicarbonate)
Tumor Lysis Labs	• Phosphorus • Uric acid • Electrolytes (sodium, potassium, chloride, and carbon dioxide)	• LDH* • Creatinine • Calcium
Coagulation	• Fibrinogen	
Urinalysis	• Color • Specific gravity • pH • Bilirubin • Ketones • Glucose • Occult Blood (Hemoglobin) • Leukocyte esterase	• Protein • Urobilinogen • Nitrites Microscopic • White blood cells (WBCs) • Red blood cells (RBCs) • Casts, crystals, bacteria
Cardiac Tests	• 12-lead Electrocardiogram (ECG)	• Echocardiogram (ECHO) or Multigated Acquisition (MUGA) scan
Pharmacodynamic Tests	• MCL-1 dependence by mitochondrial profiling in bone marrow	
Other Tests	• Pregnancy test (urine or serum determination of β-hCG in females of childbearing potential)	• Chest radiograph

*Recommended every 24 hours in patients receiving alvocidib.

APPENDIX E – RESPONSE CRITERIA

Response to treatment will be evaluated according to different criteria depending on the stage of the study.

During Stage 1, responses will be defined by the International Working Group and 2010 European LeukemiaNet Criteria.

During Stage 2, responses will be defined by the 2017 European LeukemiaNet Criteria.

INTERNATIONAL WORKING GROUP CRITERIA

Response Criteria in AML

Response Criterion	Time of Assessment	Neutrophils (/μL)	Platelets (/μL)	Bone Marrow Blasts (%)	Other
Early treatment assessment	7-10 days after therapy	NA	NA	< 5	
Morphologic leukemia-free state	Varies by protocol	NA	NA	< 5	Flow cytometry EMD
Morphologic CR	Varies by protocol	> 1,000	> 100,000	< 5	Transfusion EMD
Cytogenetic CR	Varies by protocol	> 1,000	> 100,000	< 5	Cytogenics—normal, EMD
Molecular CR	Varies by protocol	> 1,000	> 100,000	< 5	Molecular—negative, EMD
Partial remission	Varies by protocol	> 1,000	> 100,000	> 50 or decrease to 5-25	Blasts < 5% if Auer rod positive

Abbreviations: AML, acute myelogenous leukemia; EMD, extramedullary disease; CR, complete remission.

Definition of Treatment Outcomes

Outcome	Response Category	Point of Measurement	Definition
Overall survival	All patients	Entry onto trial	Death from any cause
Relapse-free survival	CR	Leukemia-free state	Disease relapse or patient death from any cause
Event-free survival	All patients*	Entry onto trial	Treatment failure, disease relapse, or patient death from any cause
Remission duration	CR	Date of CR	Disease relapse

NOTE. Complete blood counts should be evaluated at least monthly, or more often if clinically indicated, to establish the durability of responses.
Abbreviations: AML, acute myelogenous leukemia; CR, complete remission.
*Under circumstances where presentation of event-free survival may be appropriate for responders only, this point should be clearly stated.

Source: Cheson BD, et al. J Clin Oncol 2003;21:4642-4649.

2010 EUROPEAN LEUKEMIANET CRITERIA

Response Criteria in AML

Category	Definition
Complete remission (CR)*	Bone marrow blasts < 5%; absence of blasts with Auer rods; absence of extramedullary disease; absolute neutrophil count > 1.0×10^9/L (1000/μL); platelet count > 100×10^9/L (100 000/μL); independence of red cell transfusions
CR with incomplete recovery (CRi)†	All CR criteria except for residual neutropenia (< 1.0×10^9/L [1000/μL]) or thrombocytopenia (< 100×10^9/L [100 000/μL])
Morphologic leukemia-free state‡	Bone marrow blasts < 5%; absence of blasts with Auer rods; absence of extramedullary disease; no hematologic recovery required
Partial remission (PR)	Relevant in the setting of phase 1 and 2 clinical trials only; all hematologic criteria of CR; decrease of bone marrow blast percentage to 5% to 25%; and decrease of pretreatment bone marrow blast percentage by at least 50%
Cytogenetic CR (CRc)§	Reversion to a normal karyotype at the time of morphologic CR (or CRi) in cases with an abnormal karyotype at the time of diagnosis; based on the evaluation of 20 metaphase cells from bone marrow
Molecular CR (CRm)‖	No standard definition; depends on molecular target
Treatment failure	
Resistant disease (RD)	Failure to achieve CR or CRi (general practice; phase 2/3 trials), or failure to achieve CR, CRi, or PR (phase 1 trials); only includes patients surviving ≥ 7 days following completion of initial treatment, with evidence of persistent leukemia by blood and/or bone marrow examination
Death in aplasia	Deaths occurring ≥ 7 days following completion of initial treatment while cytopenic; with an aplastic or hypoplastic bone marrow obtained within 7 days of death, without evidence of persistent leukemia
Death from indeterminate cause	Deaths occurring before completion of therapy, or < 7 days following its completion; or deaths occurring ≥ 7 days following completion of initial therapy with no blasts in the blood, but no bone marrow examination available
Relapse¶	Bone marrow blasts ≥ 5%; or reappearance of blasts in the blood; or development of extramedullary disease

Definitions of response criteria are based primarily on those given by Cheson et al.[2]

*All criteria need to be fulfilled; marrow evaluation should be based on a count of 200 nucleated cells in an aspirate with spicules; if ambiguous, consider repeat exam after 5 to 7 days; flow cytometric evaluation may help to distinguish between persistent leukemia and regenerating normal marrow; a marrow biopsy should be performed in cases of dry tap, or if no spicules are obtained; no minimum duration of response required.

†The criterion of CRi is of value in protocols using intensified induction or double induction strategies, in which hematologic recovery is not awaited, but intensive therapy will be continued. In such protocols, CR may even not be achieved in the course of the entire treatment plan. In these instances, the overall remission rate should include CR and CRi patients. Some patients may not achieve complete hematologic recovery upon longer observation times.

‡This category may be useful in the clinical development of novel agents within phase 1 clinical trials, in which a transient morphologic leukemia-free state may be achieved at the time of early response assessment.

§Four studies showed that failure to convert to a normal karyotype at the time of CR predicts inferior outcome.[112-115]

‖As an example, in CBF AML low-level PCR-positivity can be detected in patients even in long-term remission. Normalizing to 10^4 copies of *ABL1* in accordance with standardized criteria, transcript levels below 12 to 10 copies appear to be predictive for long-term remission.[108-110]

¶In cases with low blast percentages (5-10%), a repeat marrow should be performed to confirm relapse. Appearance of new dysplastic changes should be closely monitored for emerging relapse. In a patient who has been recently treated, dysplasia or a transient increase in blasts may reflect a chemotherapy effect and recovery of hematopoiesis. Cytogenetics should be tested to distinguish true relapse from therapy-related MDS/AML.

Source: Döhner H, et al. Blood 2010;115:453-474.

2017 EUROPEAN LEUKEMIANET CRITERIA

Category	Definition	Comment
Response		
CR without minimal residual disease (CR_{MRD-})	If studied pretreatment, CR with negativity for a genetic marker by RT-qPCR, or CR with negativity by MFC	Sensitivities vary by marker tested, and by method used; therefore, test used and sensitivity of the assay should be reported; analyses should be done in experienced laboratories (centralized diagnostics)
Complete remission (CR)	Bone marrow blasts <5%; absence of circulating blasts and blasts with Auer rods; absence of extramedullary disease; ANC ≥1.0 × 10^9/L (1000/μL); platelet count ≥100 × 10^9/L (100 000/μL)	MRD^+ or unknown
CR with incomplete hematologic recovery (CR_i)	All CR criteria except for residual neutropenia (<1.0 × 10^9/L [1000/μL]) or thrombocytopenia (<100 × 10^9/L [100 000/μL])	
Morphologic leukemia-free state (MLFS)	Bone marrow blasts <5%; absence of blasts with Auer rods; absence of extramedullary disease; no hematologic recovery required	Marrow should not merely be "aplastic"; at least 200 cells should be enumerated or cellularity should be at least 10%
Partial remission (PR)	All hematologic criteria of CR; decrease of bone marrow blast percentage to 5% to 25%; and decrease of pretreatment bone marrow blast percentage by at least 50%	Especially important in the context of phase 1-2 clinical trials
Treatment failure		
Primary refractory disease	No CR or CR_i after 2 courses of intensive induction treatment; excluding patients with death in aplasia or death due to indeterminate cause	Regimens containing higher doses of cytarabine (see Table 8) are generally considered as the best option for patients not responding to a first cycle of 7+3; the likelihood of responding to such regimens is lower after failure of a first
Death in aplasia	Deaths occurring ≥7 d following completion of initial treatment while cytopenic; with an aplastic or hypoplastic bone marrow obtained within 7 d of death, without evidence of persistent leukemia	
Death from indeterminate cause	Deaths occurring before completion of therapy, or <7 d following its completion; or deaths occurring ≥7 d following completion of initial therapy with no blasts in the blood, but no bone marrow examination available	
Response criteria for clinical trials only		
Stable disease	Absence of CR_{MRD-}, CR, CR_i, PR, MLFS; and criteria for PD not met	Period of stable disease should last at least 3 mo
Progressive disease (PD)*,†	Evidence for an increase in bone marrow blast percentage and/or increase of absolute blast counts in the blood: • >50% increase in marrow blasts over baseline (a minimum 15% point increase is required in cases with <30% blasts at baseline; or persistent marrow blast percentage of >70% over at least 3 mo; without at least a 100% improvement in ANC to an absolute level (>0.5 × 10^9/L [500/μL], and/or platelet count to >50 × 10^9/L [50 000/μL] nontransfused); or • >50% increase in peripheral blasts (WBC × % blasts) to >25 × 10^9/L (>25 000/μL) (in the absence of differentiation syndrome)†; or • New extramedullary disease	Category mainly applies for older patient given low-intensity or single-agent "targeted therapies" in clinical trials In general, at least 2 cycles of a novel agent should be administered Some protocols may require blast increase in 2 consecutive marrow assessments at least 4 wk apart; the date of progression should then be defined as of the first observation date Some protocols may allow transient addition of hydroxyurea to lower blast counts "Progressive disease" is usually accompanied by a decline in ANC and platelets and increased transfusion requirement and decline in performance status or increase in symptoms
Relapse		
Hematologic relapse (after CR_{MRD-}, CR, CR_i)	Bone marrow blasts ≥5%; or reappearance of blasts in the blood; or development of extramedullary disease	
Molecular relapse (after CR_{MRD-})	If studied pretreatment, reoccurrence of MRD as assessed by RT-qPCR or by MFC	Test applied, sensitivity of the assay, and cutoff values used must be reported; analyses should be done in experienced laboratories (centralized diagnostics)

ANC, absolute neutrophil count; IDH, isocitrate dehydrogenase; MLFS, morphologic leukemia-free state; WBC, white blood cell.

*The authors acknowledge that this new provisional category is arbitrarily defined; the category aims at harmonizing the various definitions used in different clinical trials.

†Certain targeted therapies, for example, those inhibiting mutant IDH proteins, may cause a differentiation syndrome, that is, a transient increase in the percentage of bone marrow blasts and an absolute increase in blood blasts; in the setting of therapy with such compounds, an increase in blasts may not necessarily indicate PD.

Source: Döhner H, Estey EH, Grimwade D, et al. Blood 2017;129(4):424–447.

APPENDIX F – MULTIVARIATE ANALYSIS AND RISK SCORE PREDICTION MODEL FOR DEVELOPMENT OF TUMOR LYSIS SYNDROME IN AML

Co-variate	*Unfavorable categories*	*Regression coefficient*	*CTLS*	*p value*	*Score**
			Odds ratio (95% CI)		
WBC	≤25×10³/L	1.1	1	<0.001	0
	25-75×10³/L		2.7 (1.4-5.4)		1
	>75×10³/L		7.3 (2.0-29.1)		2
Uric acid	≤7.5 mg/dL	2.2	1	<0.001	0
	>7.5 mg/dL		9.1 (3.3-26.6)		1
LDH	≤1×ULN	1.2	1	0.005	0
	1-4×ULN		3.9 (1.5-10.8)		1
	>4×ULN		15.2 (2.2-96.8)		2

CTLS = clinical tumor lysis syndrome; LDH = lactate dehydrogenase; ULN = upper limit of normal; WBC = white blood cells

*Patients with an overall score of ≥2 will be considered at high risk for development of TLS in this study.

Source: Montesinos P, Lorenzo I, Martín G, et al. Haematologica 2008;93(1):67–74.

APPENDIX G –ASSESSMENT OF PRIOR AND CUMULATIVE ANTHRACYCLINE/ANTHRACENEDIONE EXPOSURE*

	Cumulative Dose Conversion				**Dauno**rubicin isotoxic Dose Equivalent
Doxorubicin:	mg/m²	Cumulative dose	x 1.00	=	mg/m²
Daunorubicin:	mg/m²	Cumulative dose	x 1.00	=	mg/m²
Mitoxantrone:	mg/m²	Cumulative dose	x 4.00	=	mg/m²
Idarubicin:	mg/m²	Cumulative dose	x 5.00	=	mg/m²
Epirubicin:	mg/m²	Cumulative dose	x 0.67	=	mg/m²
Patients who have received more than one anthracycline should have each individual agent cumulative dose converted to **dauno**rubicin dose equivalents using these conversion factors, and then added together.					Total **Dauno**rubicin Dose Equivalent: mg/m²

SUM BELOW

* adapted from Long-Term Survivor Guidelines, Version 4.0. October, 2013 ©Children's Oncology Group